MiR-539 promotes development of colorectal cancer through alleviating expression of Aquaporin (AQP)-9 associated with glucose metabolism

Yanjun An (anyanjun2017@163.com)
Shanxi University of Traditional Chinese Medicine https://orcid.org/0000-0002-9222-7047

Jiandong Zhao
Shanxi University of Traditional Chinese Medicine

Yourui Zhang
Shanxi University of Traditional Chinese Medicine

Wen Wu
Shanxi University of Traditional Chinese Medicine

Jiangtao Hu
Shanxi University of Traditional Chinese Medicine

Haosen Hao
Shanxi University of Traditional Chinese Medicine

Yu Qiao
Shanxi University of Traditional Chinese Medicine

Ying Tao
Shanxi University of Traditional Chinese Medicine

Liping An
Shanxi University of Traditional Chinese Medicine

Research

Keywords: proliferation, apoptosis, colon cancer, aquaporin (AQP), glucose metabolism, microRNA

Posted Date: May 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-25024/v1

Abstract

Background

AQP-9 is the key element of apoptosis, but its mechanism related to glucose metabolism in colon cancer was unclear. Therefore, the role of miR-539 in regulating expression of AQP-9 and its bio-functions were investigated.

Methods

The target gene of miR-539 was verified. The cell growth, apoptosis, and proliferation of SW480 regulated by AQP-9 and miR-539 were explored. The proteins expression associated with glucose metabolism pathway and apoptosis-related proteins were determined. Moreover, the glucose oxidase colorimetric method was used to measure the production of glucose in SW480.

Results

AQP9 was the target gene of miR-539. Interference on AQP-9 expression inhibited cell proliferation and growth of SW480, promoted its apoptosis by regulating Bcl-2 family members. Meanwhile, it caused down-expression of GOT1, GK and G6PC. Through inhibiting the expression of AQP-9 in SW480 could reduced cellular glucose production process. The above bio-functions could be achieved by utilization of miR-539 to suppress the expression of AQP-9, too.

Conclusion

Our work identified that the utilization of siRNA of AQP-9 with miR-539 could cause inhibition of proliferation, growth and induce apoptosis in SW480, which was associated with gluconeogenesis genes GOT1, GK, and G6PC in the progression of cellular glucose metabolism.

Introduction

Colorectal cancer (CRC) is the second most common type of cancer in the world, resulting in approximately 600,000 deaths each year [1]. About 90% are sporadic CRC, and the remaining are family hereditary CRC. Genetic mutations and inflammatory response are the major reasons for the tumorigenesis of CRC [2]. Due to its difficult early diagnosis, easy early metastasis, rapid development and lack of effective therapy, the five-year survival of patients with distant metastasis was about 14.2% [1, 3, 4]. The pathogenesis of CRC may be related to instability of genome and/or instability status of epigenome, etc [4, 5].

The vital channel-forming protein family, AQPs (aquaporins) are the key factor involved in function of channels for water [6]. Until now, 10 AQPs were confirmed [7], which were divided into two subgroups based on their bio-functions. They are all selective aquaglyceroporins, glycerol and water could be transported through these channels [8]. AQP-9 is not only permeable to water, but also none charged solutes, such as lactate. The accumulation of lactate in cancer cells is a common charecteristics, meanwhile the uptake of glucose increase, especially in Warburg Effect or aerobic glycolysis in normoxic conditions [9].

AQP-9 expression may explain their conflict to ischemic and hypoxic conditions in brain tumor, by facilitating clearance of glycerol [10] and lactate [11] resulting from cellular damage and hypoxia, respectively [12, 13]. Hence, AQP-9 might play a vital role in both provide enlarged tolerance for hypoxia below pathological conditions and the energy metabolism in normal brain tissue. The morbidity and mortality of malignant tumors in brain is closely related to the formation of oedema involved in AQP-9, which is determined firstly in tissue of adipose, and then leukocytes [14], brain, spleen, and liver [15, 16]. AQPs are identified the promising target for treatment of cancers in recently [17–19].

Thus, the bio-functions induced by AQP-9 expression in colon cancer cell line SW480 were researched. The cell strain of human colon cancer cell line SW480, which is widely used to search after the molecular pathogenesis of CRC. The aim of the present study was to investigate the effect of AQP-9 on bio-functions in CRC cells and CRC development associated with glucose metabolism.

Materials And Methods

Cell line of SW480

The human colon cell line SW480 was obtained from ATCC (American Type Culture Collection), and cultured with DMEM medium (Gibco, Carlsbad, CA, USA) and 10% FBS (Gibco, Carlsbad, CA, USA) in a humidified incubator with 95% air, 5% CO_{2-} humidified atmosphere at 37℃.

This research was ethically approved by the Ethics Committee of Institute of Shanxi Traditional Chinese Medicine, Hospital of Shanxi Traditional Chinese Medicine on the basis of the Declaration of Helsinki. We clearly confirmed that informed consents were obtained from all patients. All informed consent documents were obtained in writing from all participants and then kept in our hospital. Moreover, the ethical approval number was 0015–0047.

Constructure of plasmid

The plasmids of AQP-9, which could be over expression, was generated using standard techniques with pCMV-4 plasmids and verified by sequencing. Then, a total of 4×10^5 SW480 cells cultured in 6-well plates, and transfected with plasmids (4 μg) in each well using Lipofectamine 2000.

Western Blot Analysis

The total protein from colon cells was extracted using RIPA lysis buffer, then concentrations of total proteins were quantified with bicinchoninic acid assay. Total protein (50 μg) was added into 12% SDS-PAGE gel, further transferred onto polyvinylidene difluoride membranes. 5% non-fat dry milk in Tween-20 (0.1%)-containing TBS was used to block the membranes, then which was incubated with primary antibodies against AQP-9 (1:1000; sc-14988; Santa Cruz Technologies Inc., Santa Cruz, CA), G6PC (1:2000; sc-420443; Santa Cruz Technologies Inc., Santa Cruz, CA), GK (1:1500; sc-55496; Santa Cruz Technologies Inc., Santa Cruz, CA), GOT1 (1:1500; ab 220871; Abcam, Cambridge, UK), anti-Bax (1:800; sc-493; Santa Cruz, dallas, TX, USA), anti-Bcl-2 (1:1200; sc-126; Santa Cruz, dallas, TX, USA) and anti-β-ctin (1:1000; 5174; CST, Danvers, MA, USA), further incubated using the HRP (horseradish peroxidase)-conjugated second antibodies (1:1000; Cell Signaling Technology, Beverly, MA, USA). Blots were visualized by electrochemiluminescence detection system (Thermo Fisher Scientific, Waltham, MA, USA).

The production of glucose

The productions of glucose in SW480 cells were detected with glucose oxidase colorimetric method. The gluconeogenic medium was used to incubated for the isolated SW480 cells for 4 h [20].

Analyze viability of SW480 cells

The viability of SW480 cells was detected using CCK-8 method. Briefly, cultured primary cortical neurons were cultured in plates (5×10^3 cells/well). After 24 hrs of incubation, CCK-8 solutions were added into each well. After incubation for another 16 hrs, and then absorbance (450 nm) was measured with Microplate Reader.

Determine proliferation of SW480 cells

The proliferation of SW480 cells were detected with XTT (sodium 3'-[1-(phenylaminocarbonyl)-3, 4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate) (Roche Applied Science, Mannheim, Germany). After cultured in 96-well plates (3×10^3 cells/well) for 48 hrs, SW480 cells were dealt with plasmids or siRNA, then cell proliferation assay was performed, the absorbance (450 nm) was measured with microtiter plate reader (Bio-Rad)

BrdU assay and determine of mitotic entry

The transfection of siRNA or plasmids was conducted during the interval of two blocks with thyidine for avoid the its potential effect on cell cycle. The synthesis of DNA was determined with BrdU labeling, BrdU-positive SW480 cells were manually scored with immunofluorescence microscope. Furthermore, according to DNA staining using time-lapse videomicroscopy, the events of mitotic entry were recorded through observing condensation of DNA and morphology of nucleus.

Determimation of apoptosis

Apoptotic rates of cells were determined with FITC Annexin-V Apoptosis Detection Kit I (BD, San Diego, California, USA). Briefly, cells were collected, washed with PBS and resuspended in binding buffer, double-

stained with propidium iodide (PI) and Annexin V-FITC, followed by the apoptosis analysis using flow cytometry (BD Biosciences, San Jose, CA, USA).

Quantitative reverse transcription (qRT) PCR

RNA was extracted from cells using Trizol (Invitrogen, USA) according to the manufacturer's instructions. The target miRNA was obtained with mirVanaTM miRNA Isolation Kit (Invitrogen) from SW480 cells. The reverse transcription of miR-539-5p was performed based on All-inOneTM miRNA RT-qPCR Detection Kit (GeneCopoeia Inc., Rockville, MD, USA). QRT-PCR analysis was performed with TIANScript RT KIT (Tiangen biotechnology co. LTD, China) on a ABI7500 (Applied Biosystems, USA). The expression of miR-539 was normalized using U6 (forward: CTCGCTTCGGCAGCACA, reverse: AACGCTTCACGAATTTGCGT), and the primers of miR-539-5p was GGAGAAAUUAUCCUUGGUGUGU. Each reaction was run in triplicate, and the relative expression of each targeted gene was determined using $2^{-\triangle\triangle CT}$ comparative method and normalized with control of GAPDH.

Dual-luciferase reporter gene assay

The fragment at 3'-UTR region of AQP-9 with potential miR-539 binding site was cloned into pmirGlo vector (GenePharm) containing luciferase gene. Meanwhile, the mutation vector of AQP-9 (AQP-9 mut) was used as control. Dual-Luciferase Reporter Assay System (Promega Biotech Co., Madison, WI, USA) was used to assess the activities of luciferase in SW480 treated with mimics of miR-539 or its inhibitors, which were all purchased form GenePharma (Shanghai, P.R. China). The scrambled negative control RNA (miR-NC) were used as control.

Statistics

The continuous variables with normally distribute were indicated with mean ± SD (standard deviation). GraphPad Prism 8.0 was used to conduct statistical analysis. For comparisons between two groups, Student's *t*-test was performed. Moreover, one-way analysis of variance (ANOVA) with Kruskal-Wallis post hoc tests were performed for multiple group comparisons to compare and analyze quantitative data. Besides, abnormally distributed data between two groups were analyzed with Kruskal-Wallis analysis of variance method. $P < 0.05$ was considered as difference with statistically significance.

Results

AQP9 was the target of miR-539

The online software TargetScan (targetscan.org/, version 7.2) was used to predict the target gene of miR-539. The luciferase activity assay was performed, and then AQP9 was verified the potential aim of miR-539 (Fig. 1A and 1B). It meant that miR-539 could straightly bind the 3'-UTR of AQP9. Moreover, the protein expression of AQP9 down-regulated by miR-539 (Fig. 1C). The results demonstrated the mRNA of AQP9 was the target gene of miR-539. Then, miR-539 was used as the siRNA of AQP9.

The protein expression of GOT1, GK, G6PC, and AQP-9 in SW480 cells

The expression level of GOT1, GK, G6PC, and AQP-9 proteins in SW480 cells treated with over-expression AQP-9 were higher significantly than SW480 cells (control). In siRNA of AQP-9 group, the expression level of GOT1, GK, G6PC, and AQP-9 proteins decreased slightly compared to control cells (Fig. 2). It demonstrated that over-expression of AQP-9 could promote the expression level of GOT1, GK, G6PC, and AQP-9 proteins in SW480 cells, but siRNA of AQP-9 could reversed this phenomenon.

AQP-9 facilitated production of glucose in SW480 cells

The production of glucose in SW480 cells treated with over expression of AQP-9 obviously increased compared to control cells ($P < 0.01$). But in siRNA of AQP-9 group, the production of glucose significantly reduced compared to control cells ($P < 0.01$) (Fig. 3). These data demonstrated the production of glucose in SW480 cells was promoted by AQP-9 over-expression, but AQP-9 siRNA could reverse it.

Down regulation of AQP-9 inhibited proliferation and growth of SW480 cells

CCK-8 assay was conducted to evaluate the role of over expression or down expression of AQP-9 on growth and proliferation of SW480 cells. The absorbance of SW480 cells increased significantly after treated with pCMV-4-AQP-9 for 48 hrs ($P < 0.01$). But, the absorbance decreased obviously after treated with siRNA AQP-9 ($P < 0.01$) (Fig. 4A). Moreover, silencing of AQP-9 in SW480 cells induced remarkable growth suppression compared with control group based on XTT assays. However, over expression AQP-9 significantly enhanced growth of SW480 cells ($P < 0.01$) (Fig. 4B). It confirmed down regulation of AQP-9 expression restrained cell proliferation and growth of SW480 cells, but over expression of AQP-9 promoted it.

Down regulation of AQP-9 expression inhibited mitotic entry of SW480 cells

Based on the results of BrdU assay, the data showed that the mitotic cells of SW480 cells was obviously delayed by knockdown expression of AQP-9 (P 0.01). Reversely, there were not too much different between pCMV-4 transfected group and siRNA control transfected SW480 cells about DNA synthesis ($P > 0.05$). Moreover, up-regulation of AQP-9 expression in SW480 cells enhanced remarkably mitotic entry of cells (P 0.01) (Fig. 5A). Reversely, there were not too much different between pCMV-4 transfected group and siRNA control transfected SW480 cells about mitotic entry ($P > 0.05$). But, the mitotic entry of cells was delayed obviously after treated with siRNA AQP-9 in SW480 cells ($P < 0.01$) (Fig. 5B). It indicated that through inhibiting the expression of AQP-9 could induce mitotic entry of SW480 cells, moreover it could be enhanced through up-regulation of AQP-9 expression.

The expression of AQP-9 affected apoptosis of SW480 cells

The roles of AQP-9 over-expression or down-regulation with siRNA of AQP-9 on the distribution of cell cycle in SW480 cells were studied using flow cytometry. Over expression of AQP-9 induced significantly decrease of SW480 cell apoptosis ($P < 0.01$). Moreover, down-regulation of AQP-9 induced a significantly cell apoptosis in SW480 cells ($P < 0.01$) (Fig. 6). It indicated that through inhibiting the expression of AQP-9, cell apoptosis of SW480 cells was remarkably induced, moreover it could be enhanced through up-regulation of AQP-9 expression.

AQP-9 affected expression of apoptosis-related proteins in SW480 cells

The proteins expression of apoptosis-related gene Bcl-2 and Bax were determined in SW480 cells. The expression levels of Bcl-2 up-regulated by over expression of AQP-9, but decreased significantly in cells treated with siRNA of AQP-9. On the contrary, Bax expression in SW480 cells treated with over expression of AQP-9 decreased significantly, but obviously enhanced in SW480 cells treated with siRNA of AQP-9 (Fig. 7). It indicated that the apoptosis induced by down regulation of AQP-9 expression in SW480 cells was associated with Bcl-2 family.

Discussion

AQPs are channel proteins penetrate integral plasma membrane, and belong to MIP (major intrinsic protein). Small uncharged solutes and water can passively permeated these channels [21]. Their highly conserved NPA (asparagine-proline-alanine) boxes structure identify its AQP, meanwhile is vital to formation of pore for water-permeate. All AQPs have not only the special construction of hydrophobic repeated NPA boxes and conserved six transmembrane domains, but minimal different to each other [22, 23]. Besides, the NPA motifs have only poor conservation sequences that are AQP-like [24].

Focusing on apoptotic mechanism related to AQP-9 in SW480 cells, over expression or knockdown of AQP9 was induced. At first, the online software TargetScan (targetscan.org/, version 7.2) was used to predict the target gene of miR-539. The luciferase activity assay was performed, and then AQP9 was verified the potential aim of miR-539. Our results demonstrated the mRNA of AQP9 was the target gene of miR-539. Then, miR-539 was used as the siRNA of AQP9.

Moreover, the data confirmed that growth, proliferation, and mitotic entry of SW480 cells were suppressed by inhibiting expression of AQP-9, however, enhanced by over expression of AQP-9. Furthermore, the apoptosis of SW480 cells was induced by inhibiting expression of AQP-9, and enhanced by over expression of AQP-9. The levels of Bcl-2 increased in SW480 cells treated with over expression of AQP-9, but decreased significantly in cells treated with siRNA of AQP-9 (miR-539). On the contrary, the expression levels of Bax decreased in SW480 cells treated with over expression of AQP-9, but obviously promoted by siRNA of AQP-9 (miR-539). It indicated that the apoptosis induced by down regulation of AQP-9 expression in SW480 cells was associated with Bcl-2 family.

In recent years, there were two human models about metabolism, which could be potentially utilized for patient [25, 26]. The generic models of human metabolism were the base of context models, such as metabolism of cancer and metabolic activities [27, 28]. The vigorous exercise and fasting induced diminishment of glucose level in blood can activate cell gluconeogenesis. Accordingly, the production of glucose restore the homeostasis of glucose. The induction of gluconeogenesis can be achieved by stimulus of hormone that is associated with four gluconeogenesis in these main reactions at transcriptional, post-translational level, even allosteric alterations. The following enzymes of G6Pase (glucose-6-phosphatase), F1, 6BPase (fructose-1, 6-bisphosphatase), PEPCK (phosphoenolpyruvate carboxykinase), and PC (pyruvate carboxylase) can catalyze these reactions. It is critical effect on induction of gluconeogenesis come from these enzymes encoded by these gene through transcriptional regulation [29].

Therefore, focus on the connection between metabolism of glucose and AQP-9, which may be related to CRC tumorigenesis, the investigation about the role of AQP-9 in enhancing de novo synthesis of glucose, gluconeogenesis was conducted. In an attempt to comprehensively confirm the pathways of metabolism associated with AQP-9, over expression and down regulation of AQP-9 were induced in cell line SW480, in the meanwhile, the cell functions and expression levels of these glucogenic gene was studied. We identified that up-regulation of AQP-9 could induce increased expression level of GOT1, GK, and G6PC proteins, but siRNA of AQP-9 (miR-539) reduced these expression.

It had evidences that miR-539 could induce suppression of EMT (Epithelial–Mesenchymal Transition), migration and invasion of prostate cancer cells [30]. Cao et al. Thought that miR-539 might be involved in the progression of ESCC (Esophageal Cancer Cells) and could be a new therapeutic target for this disease [31]. But, in this study, our results indicated that miR-539 promotes development of colorectal cancer through alleviating expression of AQP-9 associated with glucose metabolism.

The aims of this study were to explore associated with glucose metabolism and the methods for treatment on CRC related to AQP-9. For the purpose of identifying the pathways of metabolism at full-scale, which was related to the role of AQP-9 for treatment on CRC, the expression of glucogenic gene was examined. We performed the research on glucose metabolism and the methods for treatment on CRC related to the role of AQP-9 in enhancing de novo glucose synthesis or gluconeogenesis. Our results confirmed that AQP-9 could affect on the genes expression associated with gluconeogenesis. Consistent with these above results, the production of glucose was caused through AQP-9 over-expression in SW480 cells, however, it could be reversed through down expression with AQP-9 siRNA (miR-539).

Our study indicated through knockdown regulation of AQP-9 could cause proliferation and growth suppression of SW480 cells, which was associated with metabolic pathway involved in gluconeogenesis gene GOT1, GK, and G6PC. These genes could enhance the metabolism of glucose. In conclusion, AQP-9 can be served as the potential novel target for treatment on CRC .

Declarations

Ethics approval and consent to participate

This research was ethically approved by the Ethics Committee of Institute of Shanxi Traditional Chinese Medicine, Hospital of Shanxi Traditional Chinese Medicine on the basis of the Declaration of Helsinki. We clearly confirmed that informed consents were obtained from all patients. All informed consent documents were obtained in writing from all participants and then kept in our hospital. Moreover, the ethical approval number was 0015-0047.

Consent for publication

Not applicable.

Availability of data and material

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declares that we have no conflict of interest.

Funding

Not applicable.

Authors' contributions

YJA and JDZ contributed to the conception of the study. YJA, JDZ and YRZ contributed significantly to analysis and manuscript preparation; WW, JTH, and HSH performed the data analyses and wrote the manuscript; YJA and YQ, YT, LPA helped perform the analysis with constructive discussions.

Acknowledgements

Not applicable.

References

1. Peng H, Pan X, Su Q, Zhu LS, Ma GD. MiR-372-3p promotes tumor progression by targeting LATS2 in colorectal cancer. Eur Rev Med Pharmacol Sci. 2019;23:8332–44.
2. Gupta J, Del BBI, Igea A, Sakellariou S, Pateras IS, Gorgoulis VG, Nebreda AR. Dual function of p38alpha MAPK in colon cancer: suppression of colitis-associated tumor initiation but requirement for cancer cell survival. Cancer Cell. 2014;25:484–500.
3. Stormark K, Krarup PM, Sjövall A, Søreide K, Kvaløy JT, Nordholm-Carstensen A, Nedrebø BS, Kørner H. Anastomotic leak after surgery for colon cancer and effect on long-term survival. Colorectal Dis

2020 Feb 3. doi: 10.1111/codi.14999. [Epub ahead of print].

4. Fleming M, Ravula S, Tatishchev SF, Wang HL. Colorectal carcinoma: Pathologic aspects. J Gastrointest Oncol. 2012;3:153–73.
5. Grady WM, Markowitz SD. The molecular pathogenesis of colorectal cancer and its potential application to colorectal cancer screening. Dig Dis Sci. 2015;60:762–72.
6. Engel A, Fujiyoshi Y, Agre P. The importance of aquaporin water channel protein structures. EMBO J. 2000;19:800–6.
7. Verkman AS, Mitra AK. Structure and function of aquaporin water channels. Am J Physiol. 2000;278:13–28.
8. Kuriyama H, Kawamoto S, Ishida N, Ohno I, Mita S, Matsuzawa Y, Matsubara K, Okubo K. Molecular cloning and expression of a novel human aquaporin from adipose tissue with glycerol permeability. Biochem Biophys Res Commun. 1997;241:53–5.
9. Hirschhaeuser F, Sattler UG, Mueller-Klieser W. Lactate: a metabolic key player in cancer. Cancer Res. 2011;71:6921–5.
10. Nguyen NHT, Bråthe A, Hassel B. Neuronal uptake and metabolism of glycerol and the neuronal expression of mitochondrial glycerol-3-phosphate dehydrogenase. J Neurochem. 2003;85:831–42.
11. Magistretti PJ, Pellerin L, Rothman DL, Shulman RG. Energy on demand. Science. 1999;283:496–7.
12. Badaut J, Petit JM, Brunet JF, Magistretti PJ, Charriaut-Marlangue C, Regli L. Distribution of aquaporin 9 in the adult rat brain: preferential expression in catecholaminergic neurons and in glial cells. Neuroscience. 2004;128:27–38.
13. Badaut J, Regli L. Distribution and possible roles of aquaporin 9 in the brain. Neuroscience. 2004;129:971–81.
14. Ishibashi K, Kuwahara M, Gu Y, et al. Cloning and functional expression of a new aquaporin (AQP9) abundantly expressed in the peripheral leukocytes permeable to water and urea, but not to glycerol. Biochem Biophys Res Comm. 1998;244:268–74.
15. Elkjaer M, Vajda Z, Nejsum LN, et al. Immunolocalization of AQP9 in liver, epididymis, testis, spleen, and brain. Biochem Biophys Res Commun. 2000;276:1118–28.
16. Bhattacharjee H, Carbrey J, Rosen BP, Mukhopadhyay R. Drug uptake and pharmacological modulation of drug sensitivity in leukemia by AQP9. Biochem Biophys Res Commu. 2004;322:836–41.
17. Qin YS, Bu DX, Cui YY, Zhang XY. Difference in glycerol levels between leukemia and normal bone marrow stem cells. Oncol Lett. 2014;8:169–74.
18. Huber VJ, Tsujita M, Nakada T. Aquaporins in drug discovery and pharmacotherapy. Mol Aspects Med. 2012;33:691–703.
19. Wang F, Feng XC, Li YM, Yang H, Ma TH. Aquaporins as potential drug targets. Acta Pharmacol Sin. 2006;27:395–401.

20. Beitz E. Aquaporins from pathogenic protozoan parasites: structure, function and potential for chemotherapy. Biol Cell. 2005;97:373–83.
21. Zeuthen T. How water molecules pass through aquaporins. Trends Biochem Sci. 2001;26:77–9.
22. Zardoya R. Phylogeny and evolution of the major intrinsic protein family. Biol Cell. 2005;97:397–414.
23. Verkman AS, Mitra AK. Structure and function of aquaporin water channels. Am J Physiol Renal Physiol. 2000;278:F13–28.
24. Ishibashi K. Aquaporin subfamily with unusual NPA boxes. Biochim Biophys Acta. 2006;1758:989–93.
25. Duarte NC, Becker SA, Jamshidi N, Thiele I, Mo ML, Vo TD, et al. Global reconstruction of the human metabolic network based on genomic and bibliomic data. Proc Natl Acad Sci USA. 2007;104:1777–82.
26. Ma H, Sorokin A, Mazein A, Selkov A, Selkov E, Demin O, et al. The Edinburgh human metabolic network reconstruction and its functional analysis. Mol Syst Biol. 2007;3:135.
27. Jerby L, Shlomi T, Ruppin E. Computational reconstruction of tissuespecific metabolic models: application to human liver metabolism. Mol Syst Biol. 2010;6:401.
28. Frezza C, Zheng L, Folger O, Rajagopalan KN, MacKenzie ED, Jerby L, et al. Haemoxygenase is synthetically lethal with the tumour suppressor fumaratehydratase. Nature. 2011;477:225–8.
29. Jitrapakdee S. Transcription factors and coactivators controlling nutrient and hormonal regulation of hepatic gluconeogenesis. Int J Biochem Cell Biol. 2012;44:33–45.
30. Sun B, Fan Y, Yang A, Liang L, Cao J. MicroRNA-539 functions as a tumour suppressor in prostate cancer via the TGF-β/Smad4 signalling pathway by down-regulating DLX1. J Cell Mol Med. 2019;23:5934–48.
31. Cao Z, Zheng X, Cao L, Liang N. MicroRNA-539 Inhibits the Epithelial-Mesenchymal Transition of Esophageal Cancer Cells by Twist-Related Protein 1-Mediated Modulation of Melanoma-Associated Antigen A4. Oncol Res. 2018;26:529–36.

Figures

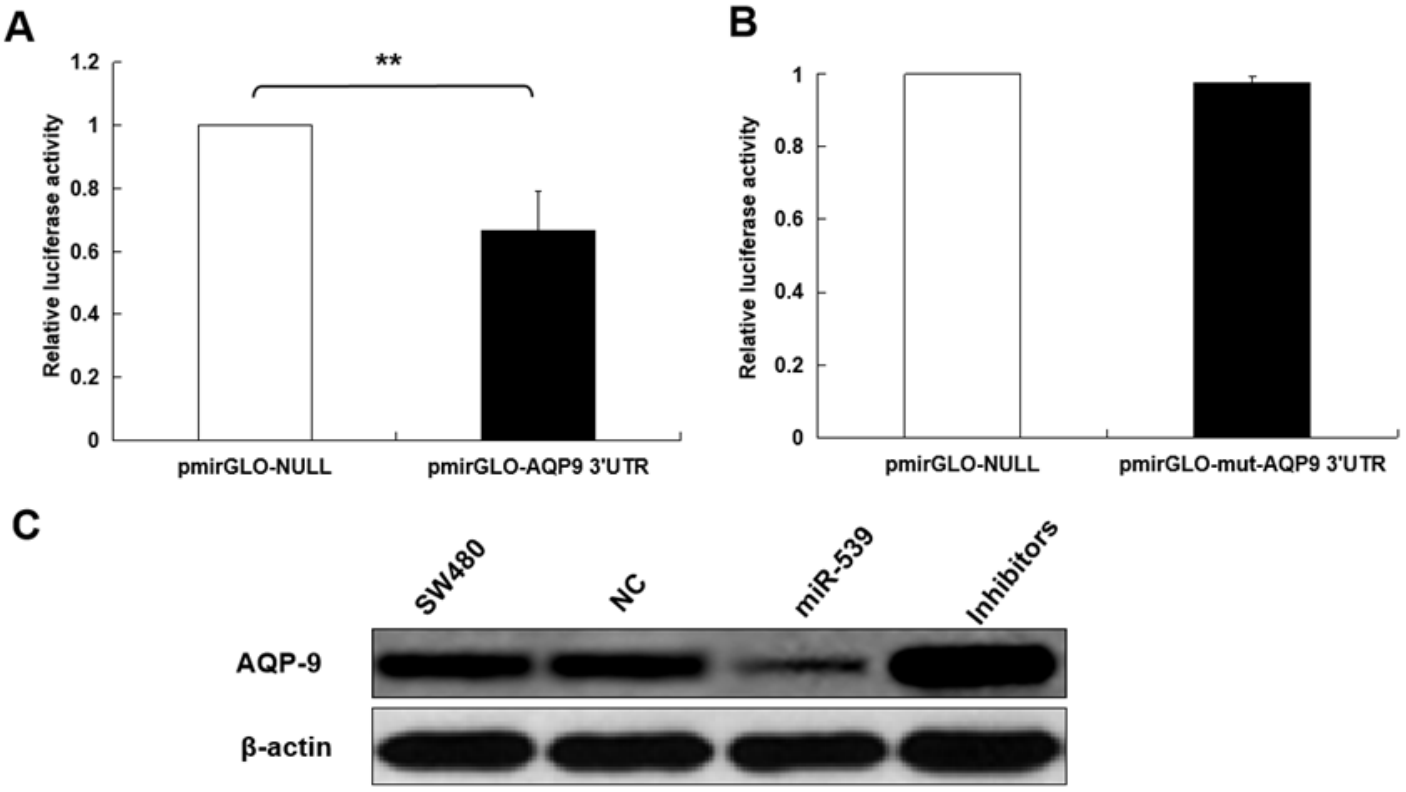

Figure 1

AQP9 was the target gene of miR-539. The online software TargetScan (targetscan.org/, version 7.2) was used to predict the target gene of miR-539. The luciferase activity assay was performed, and then AQP9 was verified the potential aim of miR-539 (Figure A and B). It meant that miR-539 could straightly bind the 3’-UTR of AQP9. Moreover, the protein expression of AQP9 down-regulated by miR-539 (Figure C). The results demonstrated the mRNA of AQP9 was the target gene of miR-539. Then, miR-539 was used as the siRNA of AQP9.

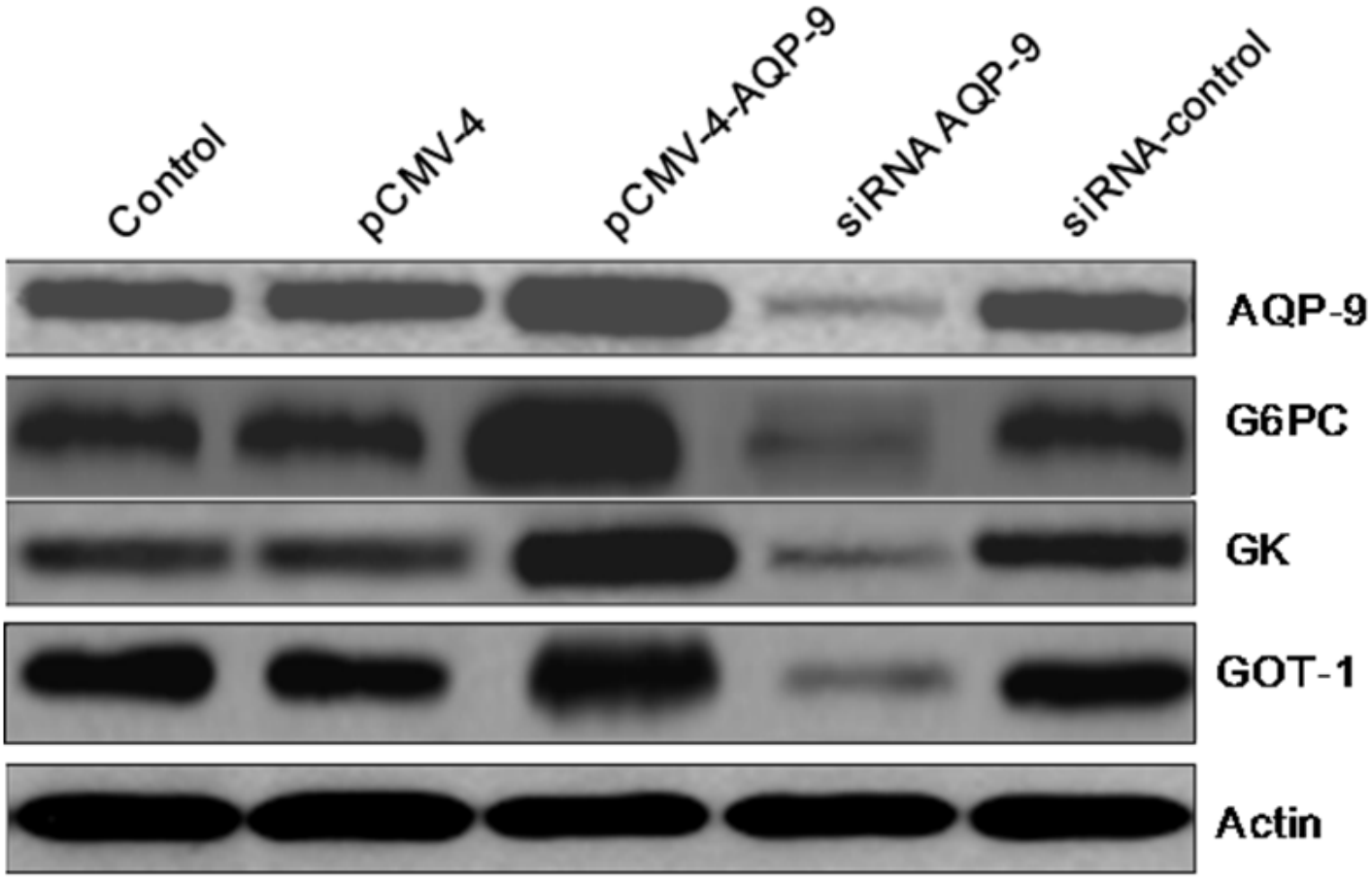

Figure 2

The expression of AQP-9, G6PC, GK and GOT1 in SW480 cells. The protein expression level of AQP-9, G6PC, GK and GOT1 in SW480 cells treated with over expression of AQP-9 increased significantly compared with control group. At siRNA AQP-9 group, the protein expression level of AQP-9, G6PC, GK and GOT1 decreased slightly compared with the control group. miR-539 was used as the siRNA of AQP9.

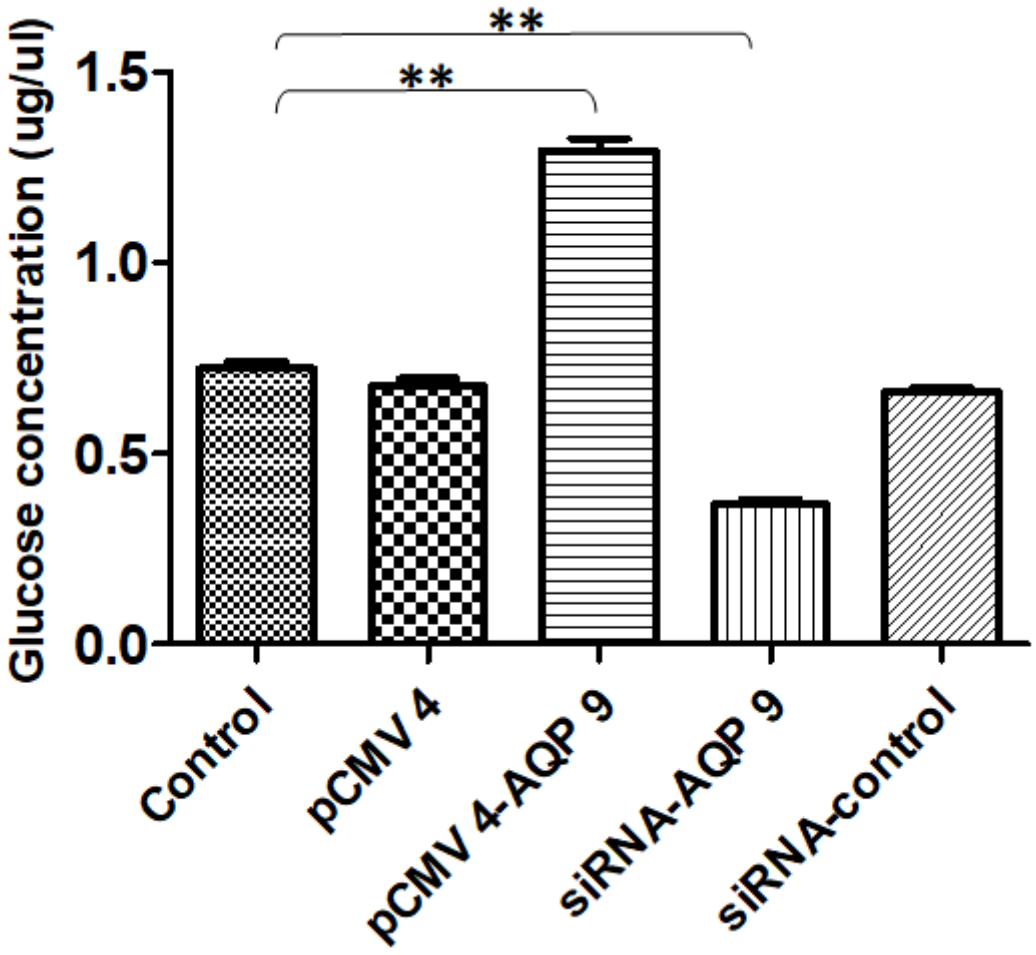

Figure 3

AQP9 enhanced glucose production in SW480 cells. The amounts of glucose production in SW480 cells treated with over expression of AQP-9 increased significantly compared with control group (P<0.01). In group of siRNA AQP-9, the amounts of glucose production decreased obviously compared with the control group (P<0.01). The data are presented as means ± SD from three independent experiments. *P<0.05, **P<0.01; P value was generated using Kruskal-Wallis ANOVA. miR-539 was used as the siRNA of AQP9.

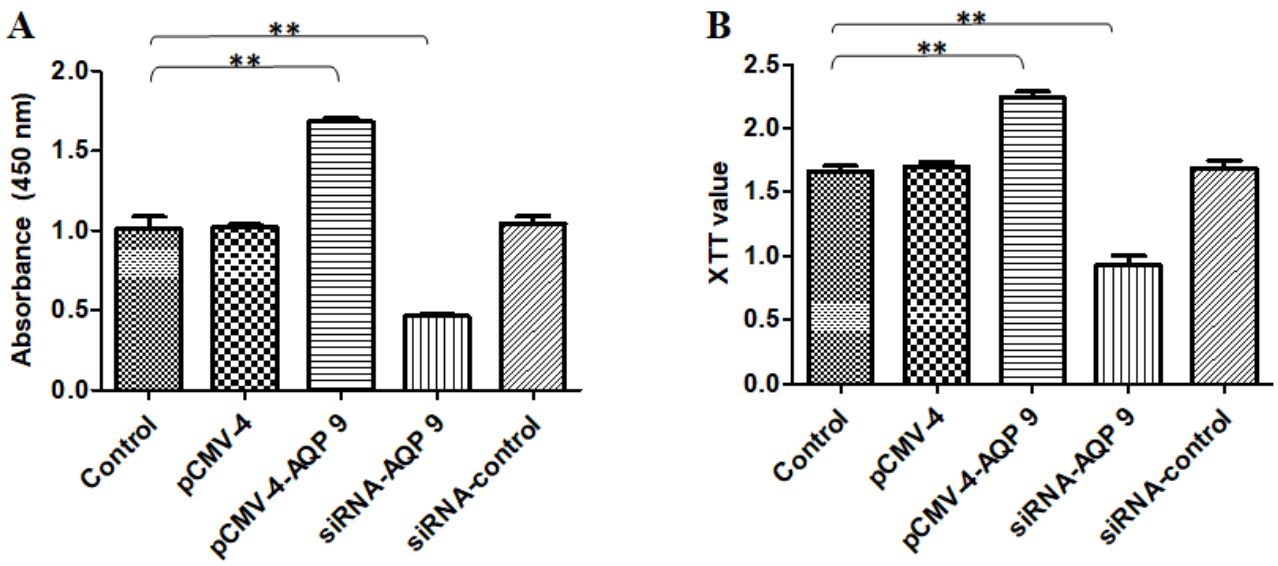

Figure 4

AQP-9 promoted proliferation and growth of SW480 cells. (A) SW480 cells (3×103) were added into plates and cultured for 24 hrs, then treated with transfection. The absorbance of SW480 cells increased significantly treated with pCMV-4-AQP-9 based on CCK-8 assay (P<0.01). But, the absorbance decreased obviously after treated with siRNA of AQP-9 (P<0.01). (B) By XTT assays, AQP9 silenced SW480 cells resulted in a significant inhibition of cell growth when compared to that of control. However, over expression AQP9 in SW480 cells significantly promoted cell growth (P<0.01). miR-539 was used as the siRNA of AQP9.

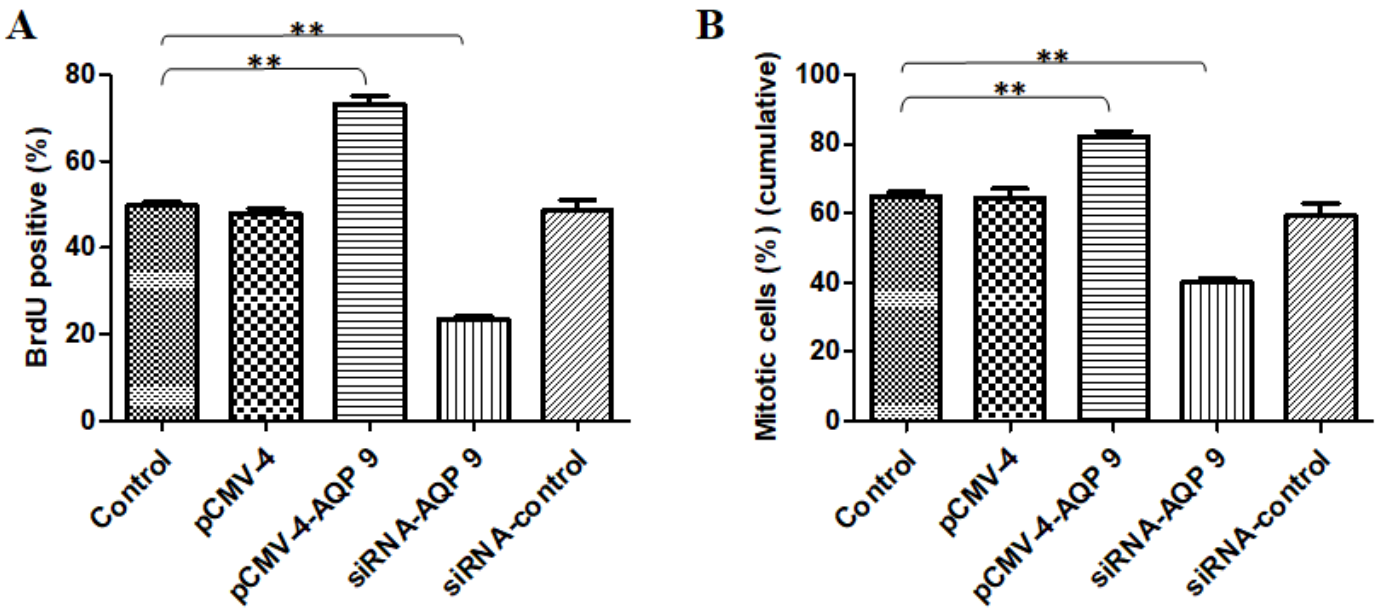

Figure 5

Down regulation of AQP9 expression inhibited mitotic entry of SW480 cells. (A) The SW480 cells was significantly delayed in AQP9-knockdown SW480 cells (P0.01) by BrdU assay. Over expression AQP9 in SW480 cells significantly promoted mitotic entry of cells (P0.01). (B) The mitotic SW480 cells was significantly promoted by over expression of AQP9 (P0.01). AQP9-knockdown significantly delayed mitotic entry of SW480 cells (P0.01). miR-539 was used as the siRNA of AQP9.

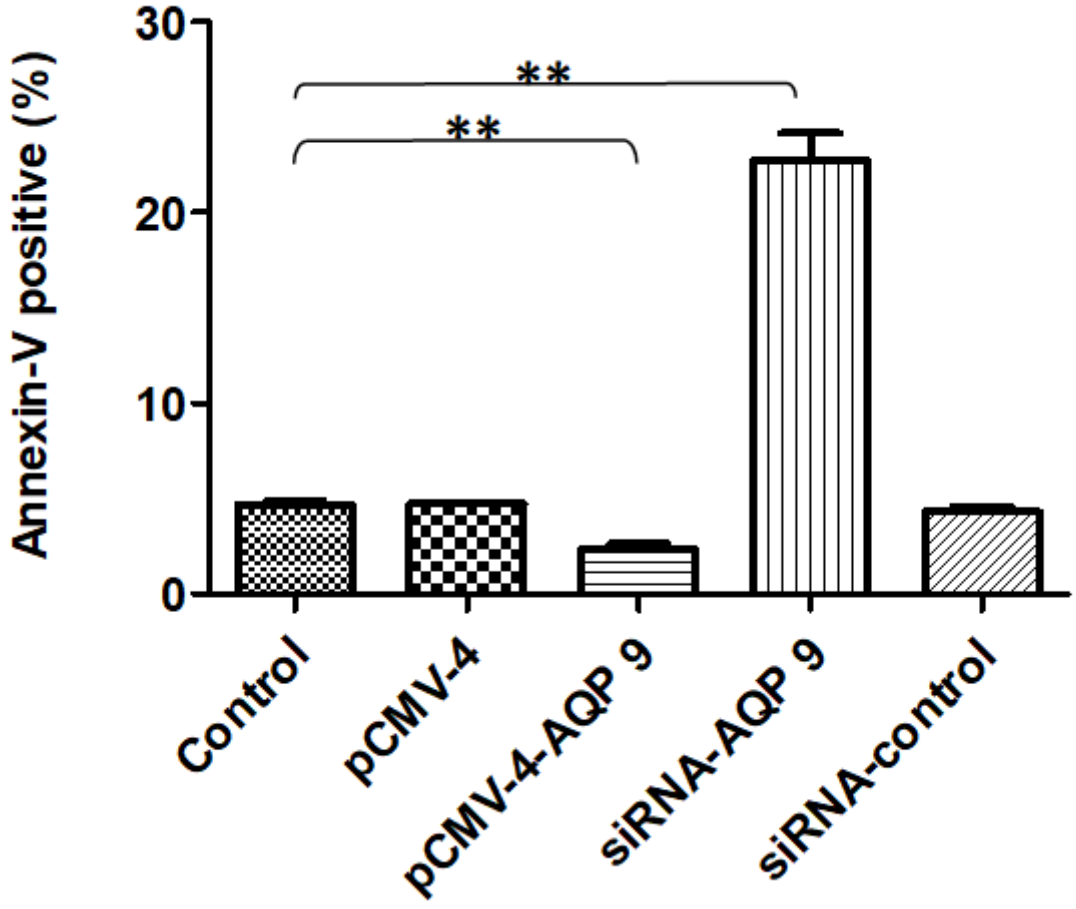

Figure 6

The expression of AQP9 affected apoptosis of SW480 cells. The effect of over expression of AQP9 or AQP9 siRNA on cell cycle distribution was determined in SW480 cells by flow cytometry. Over expression of AQP9 in SW480 cells induced decrease of apoptosis (P<0.01). Down-regulation of AQP9 in SW480 cells induced a significantly apoptosis (P<0.01). All data are represented as mean ± SEM. miR-539 was used as the siRNA of AQP9.

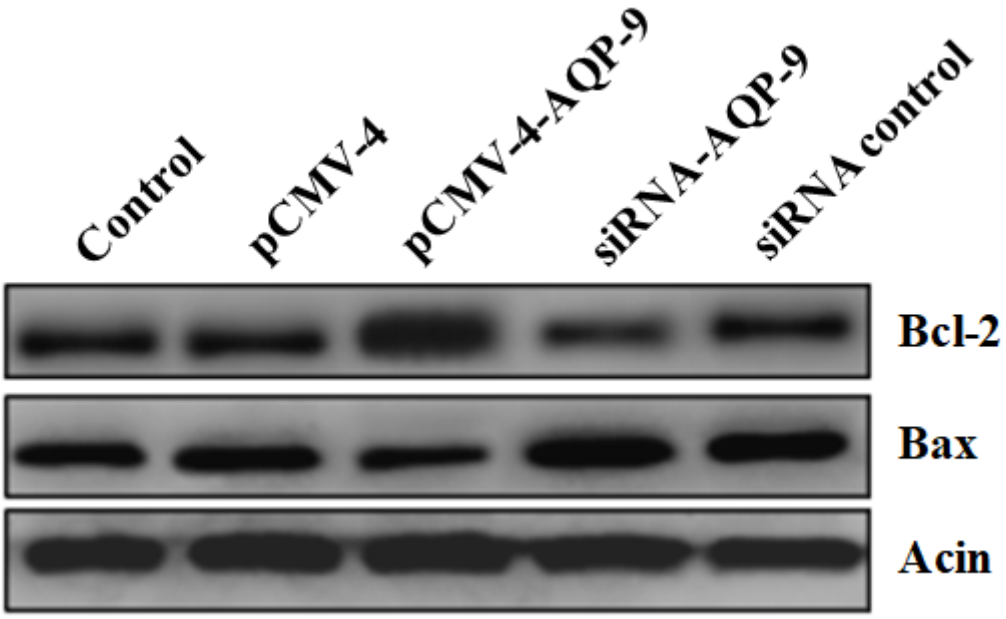

Figure 7

AQP-9 affected expression of apoptosis-related proteins of SW480 cells. The expression levels of P53, apoptosis-related proteins Bax, Bcl-2 and caspase-3 protein in SW480 cells were determined by Western Blotting. The levels of Bcl-2 increased in SW480 cells treated with over expression of AQP9, but decreased significantly in cells treated with siRNA of AQP-9. On the contrary, the levels of Bax decreased in SW480 cells treated with over expression of AQP9, but increased significantly in cells treated with siRNA of AQP-9. miR-539 was used as the siRNA of AQP9.