ORIGINAL RESEARCH

GPR78 Regulates Autophagy and Drug Resistance in Non-small Cell Lung Cancer

Xuqiang Liao, MM; Renzhong Cai, MM; Gao Li, MM; Fengxia Chen, MM

ABSTRACT

Context • Lung cancer is one of the most common forms of cancer. Autophagy and apoptosis play an important role in the development of lung cancer. Researchers have found upregulation of GRP78 expression in cancer cells of various types.

Objective • The study intended to explore the mechanism of G protein-coupled receptor 78 (GPR78) in regulating autophagy and drug resistance in non-small cell lung cancer (NSCLC).

Design • The research team performed a laboratory study.

Setting • The study took place in the Department of Thoracic Surgery at Hainan General Hospital of the Hainan Affiliated Hospital of Hainan Medical University in Haikou, Hainan, China.

Intervention • The research team cultured immortalized, normal, human bronchial epithelial cells C3 (HBEC3) lines and HBEC4 lines in a serum medium without keratinocytes and infected the expression of GPR78 in knockdown A549 cells using lentiviral agents. The team divided the cells into a control group and a shRNA-GPR78 group, the intervention group. The lentiviral silencing vector expressing shRNA targets human GPR78#1 and GPR78 #2aadam10.

Outcome Measures • The research team analyzed the mRNA expression of GPR78 in the NSCLC cell lines H1975, H1299, and A549 and in HBEC3 and HBEC4 using a real time-polymerase chain reaction (RT-PCR) and measured the proliferation of A549 cells at 0h, 24h, 48h, 72h, and 96h using yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The team also analyzed the migration and invasion ability of cells using wound healing and Transwell tests as well as measured the protein expression of the autophagy-related factors Beclin-1, microtubule-associated protein light chain 3-I/II (LC3-I/LC3-II), ubiquitin-binding protein p62 and c-Jun N-terminal kinase (JNK) using a Western blot test. The team also analyzed the protein expressions of caspase-9, caspase-3, and caspase-12 related to apoptosis using a Western blot. To detect the cell viability induced by cisplatin, the team used a Cell Counting Kit 8 (CCK-8) at the concentrations of 1μM, 3μM and 10μM.

Results • The mRNA expression of GPR78 in the H1975, H1299, and A549 cell lines was significantly higher than that in the HBEC3 and HBEC4 cell lines ($P < .05$). At 48h, 72h, and 96h, the A549 cell proliferation in the shRNA-GPR78 group was significantly lower than that of the control group ($P < .05$). The cell migration and invasion of cells in the shRNA-GPR78 group was significantly lower than that in the control group ($P < .05$), and the cell viability of the shRNA-GPR78 group was significantly lower than that of control group ($P < .05$). The expression of Beclin-1 and JNK protein in shRNA-GPR78 group was significantly higher than that in the control group ($P < .05$), and the expression of LC3-I/LC3-II and p62 protein in shRNA-GPR78 group was significantly lower than that in the control group ($P < .05$). The protein expressions of caspase-9, caspase-3, and caspase-12 in the shRNA-GPR78 group were significantly higher than those of the control group ($P < .05$), and the protein activities of RhoA and Rac1 in the shRNA-GPR78 group were significantly lower than those in the control group ($P < .05$).

Conclusion • NSCLC upregulated GPR78. The knockdown of GPR78 can attenuate the proliferation, migration, and invasion of NSCLC cells and increase the apoptosis and autophagy of NSCLC cells that cisplatin has induced. Therefore, targeting GPR78 may be a promising treatment strategy for NSCLC patients. (*Altern Ther Health Med.* 2023;29(1):130-136).

Xuqiang Liao, MM; Renzhong Cai, MM; Gao Li, MM; Fengxia Chen, MM, Department of Thoracic Surgery, Hainan General Hospital, Hainan Affiliated Hospital of Hainan Medical University, Haikou, Hainan, China.

Corresponding author: Gao Li, MM
E-mail: drligao@163.com
Corresponding author: Fengxia Chen, MM
E-mail: chenfengxia0073@126.com

Lung cancer is one of the most common forms of cancer. Histologically, it can be divided into non-small cell lung cancer (NSCLC) and small cell lung cancer,[1] with about 85% of cases having been diagnosed as NSCLC. In the past few decades, despite many advances in early detection and treatment, patients' five-year survival rate is still lower than 20%.[2] Therefore, further study on the pathogenesis and molecular mechanism of NSCLC is very important for patients' treatment and prognosis.

Autophagy and Apoptosis

Autophagy and apoptosis play an important role in the development of lung cancer.[3-5] Autophagy is an evolutionarily ancient and evolutionarily conserved catabolic process that engulfs cytoplasmic proteins, complexes, and organelles[6,7] and transports them to promote a lysosome-degradation pathway.[8]

Anticancer treatment of tumor cells, such as radiotherapy, chemotherapy, and targeted therapy, usually activate autophagy.[9] This, in turn, mediates autophagic cell death, possibly through overactivation of self-digestion. This cell death is considered to be type II programmed cell death, an important drug-resistance mechanism that supports the survival of tumor cells.[10]

The interaction between autophagy and apoptosis in NSCLC is complex. In fact, autophagy plays a double-edged role in carcinogenesis.[11] On the one hand, different cancer proteins, such as protein kinase B (AKT), phosphoinositide 3-kinases (PI3K), and B-cell lymphoma 1n (Bcl-1n) and the mutant tumor protein p53, always inhibit autophagy, which may prevent excessive degradation of protein in hungry or stressed tumor cells. On the other hand, the continuous activation of autophagy can lead to autophagy-programmed cell death or apoptosis.

Chemotherapy drugs usually induce apoptosis or programmed cell death, and the process plays a key role in the clinical treatment of human cancer, which many apoptosis-related genes and signal pathways regulate. Recently, researchers have suggested autophagy as a therapeutic target for cancer treatment.

GPR78

Interestingly, apoptosis and autophagy are closely related and may regulate each other positively and negatively. GPR78 is an orphan G protein-coupled receptor that is highly expressed in human placenta and brain tissue.[12] It's a molecular chaperone of the major endoplasmic reticulum, with calcium ion (Ca2+) binding ability. It also acts as a regulator of signals of endoplasmic reticulum stress.[13]

Yeh et al and Dong et al found upregulation of GRP78 expression in cancer cells of various types, including those of the urinary system, digestive system, breast, brain, and respiratory system and in osteosarcoma.[14,15] Lin et al and Xiong et al found that human cancers, such as liver cancer and bladder cancer, can upregulate GPR78 and that a knockdown of GPR78 expression can reduce the proliferation and invasion of bladder-cancer cells.[16,17]

The existence of GRP78 auto-antibodies in cancer patients' serum usually relates to a poor prognosis, because GRP78 can promote the proliferation, survival, and metastasis of tumor cells by inhibiting the apoptosis induced by endoplasmic reticulum stress.[18]

However, researchers haven't yet clarified the role and molecular mechanism of GPR78 in the occurrence and development of NSCLC.

Autophagy-related Factors

Beclin-1 is a necessary factor that is closely involved in the autophagy of mammalian cells. Activated autophagy usually accompanies the upregulation of Beclin-1. In addition, researchers have focused more and more attention on the role of the ubiquitin-binding protein p62, because it's a signal adapter of autophagy.

Microtubule-associated protein light chain 3-I/II (LC3-I/LC3-II) are two important autophagy-related proteins that change during autophagy. It is found that the autophagy of NSCLC cells transfected with shRNA-GPR78 can increase after cisplatin induction. This occurs because the silencing of GPR78 can increase the production of Beclin-1, c-Jun N-terminal kinase (JNK), and LC3-II, and at the same time, weaken the accumulation of p62, which indicates that the knockdown of GPR78 can increase the autophagy of NSCLC cells.

Bai et al found that JNK is a serine/threonine kinase that belongs to the mitogen-activated, protein kinase family, which plays an important role in autophagy, especially in autophagic cell death. It is found that common upstream signals can trigger apoptosis and autophagy. In fact, various stimuli can activate JNK, thus inducing apoptosis and autophagy in human cancer.[20]

Molecularly, activation of JNK can induce phosphorylation of Bcl-2, thus activating autophagy induced by Beclin1 and apoptosis mediated by caspase-3. In addition, the downstream target of JNK can upregulate the expression level of autophagy and apoptosis genes.

Caspase is a cysteine-dependent and aspartate-directed protease family, which plays a vital role in the signal pathway of apoptosis. It is found that caspases-8 and caspase-9 are responsible for activating external and internal apoptosis pathways, respectively, while endoplasmic reticulum stress activates caspase-12 in the middle, and it participates in endoplasmic reticulum-induced apoptosis.

Current Study

The current study intended to explore the mechanism of GPR78 in regulating autophagy and drug resistance in NSCLC.

METHODS

Cell Cultures

The research team performed a laboratory study that took place in the Department of Thoracic Surgery at Hainan General Hospital of the Hainan Affiliated Hospital of Hainan Medical University in Haikou, Hainan, China.

The research team purchased the immortalized, normal, human bronchial epithelial cell lines HBEC3 and HBEC4 and the cancer cell lines A549, H1975, and H1299 from the

American Type Culture Collection (ATCC). The team cultured the HBEC3 and HBEC4 cells in a keratinocyte serum medium (KSFM) from Gibco, at 37°C and 5% CO_2, and infected the expression of the GPR78 in knockdown A549 cells using lentiviral agents.

The team cultured the cancer cell lines in Dulbecco's Modified Eagle Medium (DMEM) without keratinocytes, supplemented with 10% fetal bovine serum, 100 units /ml of penicillin, 100 µg/ml of streptomycin, and 2 mM of L-glutamine at 37°C and 5% CO_2.

Procedures

The research team first evaluated the expression of GPR78 in NSCLC cell lines and normal cells and then examined the effects of the GPR78 knockdown on the growth, autophagy, and drug resistance of the NSCLC cells as well as potential molecular events. The team assumed that GPR78 played a role as an oncogene in NSCLC, and might be a target of future treatment for NSCLC patients.

Intervention. The study used the NSCLC cell line A549, and the research team divided the cells into a control group and an shRNA-GPR78 group, the intervention group. Researchers use short hairpin RNA (shRNA) to silence target gene expression via RNA interference (RNAi). The research team obtained the lentiviral silencing vector expressing shRNA that targets human GPR78#1 (TRC number: TRCN0000357332) and GPR78 #2aadam10 (TRC number: TRCN0000357333) from Sigma-Aldrich (St. Louis, Missouri, USA).

Plasmid construction and transfection. The research team amplified the full-length, DNA-coding sequence of human GPR78 (NM_080819) using the forward primer 5'-GGATCCCATGGGCCCCGGCGAG-3' and the reverse primer 5'-GTCGACGCCAGGCCCTCAGTGTG-3. The team cloned the product resulting from a real-time (RT) polymerase chain reaction (PCR) into Bacillus amyloliquefaciens (BamH1)/inositol polyphosphate 1-phosphatase (Sal1) site of the cloning vector pCMV-Tag2A construct and verified it using DNA sequencing.

The research team amplified the membrane-tethering protein P115-regulators of G protein signaling (RGS) or P115-RGS—amino acid residues 1-252—and the protein-coupled receptor kinase 2 (GRK2)-RGS—amino acid residues 54-175—using PCR and subcloned them to the BamH1/Escherichi coli 1 (EcroR1) site of the pCMV-Tag2A construct.

According to the manufacturer's instructions, the team transfected the plasmids human GPR78#1 and GPR78 #2aadam10 into cells using lipofectamine 2000 (Thermo Fisher Scientific Life Sciences, Grand Island, NE, USA).

For lentiviral production, the team co-transfected the A549 NSCLC cells with plasmids: porcine cytomegalovirus (pCMV)-vesicular stomatitis virus G (VSV-G), pCMV-dr8.2-dvpr, and shRNA. After 24 hours, the team collected the supernatant and infected the cells with the lentiviral preparation. After 24 hours of infection, the team completed the cell selection by incubating them with puromycin for another 48 hours.

RT-PCR. The research team extracted the RNA using TRIzol (Thermo Fisher Scientific Science and Technology) and quantified it using spectrophotometry, a micro-nucleic-acid protein concentration analyzer. According to the manufacturer's protocols, the team used the Superscript III reverse transcriptase (Thermo Fisher Scientific) to perform reverse transcription of RNA.

The team performed the RT-PCR using a MyiQ RT-PCR detection system. The team purchased the Taqman probes for detection of GPR78 (detection ID: Hs01574416_m1), GPR26 (Hs00538034_m1), and actin (detection ID: HS. PT.56a.21538384) from Thermo Fisher Scientific Technology. The team normalized the threshold cycle number of each gene to the threshold cycle number of actin and converted the resulting value to a linear scale.

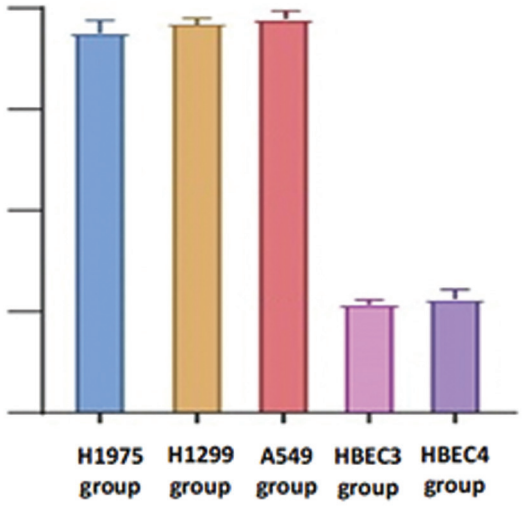
Western blot analysis. The research team extracted the total cellular proteins in cell lines using a radioimmunoprecipitation assay (RIPA) buffer (Beyotime, Shanghai, China) and a protease inhibitor at 4°C. The team then centrifuged them at 12 000 rpm for 20 minutes and collected the supernatant at 4°C. The team measured the concentrations of these protein samples using a bicinchoninic acid (BCA) protein assay kit (Beyotime, Shanghai, China); separated them, at 30 µg per lane, using sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE); and transferred them to a nitrocellulose membrane (MilliporeSigma, Burlington, MA, USA).

After sealing the protein samples with 5% skim milk, the research team incubated the membrane with the designated primary antibody from at 4 °C overnight. The next day the team incubated the membrane with the corresponding anti-mouse or anti-rabbit, immunoglobulin G (IgG) secondary antibody, coupled with horseradish peroxidase (HRP), at room temperature for one hour. The team used an Western Bright enhanced chemiluminescence (ECL) kit (Menlo Park, CA, USA) and the Chemidoc XRS+ imaging system (Bio-Rad, Hercules, CA, USA) to observe the protein bands.

MTT assay. The research team cultured the cells inoculated in 96-well plates for 0h, 24h, 48h, 72h, and 96h and then added 0.5 mg/ml of yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). After incubating the cells at 37°C for 4 hours, the team carefully absorbed the culture medium in the well to prevent the cells from forming the formazan crystals and dissolved the crystals with 100 µl of dimethyl sulfoxide (DMSO). The team used the Multiskan Spectrum spectrophotometer to measure the absorbance at 570 nm.

Cell migration and invasion test. The research team measured the Transwell migration using a 6.5-mm diameter, polycarbonate filter with an 8-micron pore size. The team inoculated the cells (4 × 104) in the top chamber that had been suspended in 100 µl of DMEM containing 0.5% FBS. The bottom chamber contained 500 µl of DMEM with 10% FBS. The team allowed the cells to migrate for 12 hours and removed the non-migrated cells with cotton swabs.

Figure 1. The mRNA Expression Level of GPR 78 in the H1975, H1299, and A549 cancer cell lines and in the immortalized, normal, human bronchial epithelial cell lines HBEC3, and HBEC4



Note: $P < .05$ indicates that the mRNA expression of GPR78 in the H1975, H1299, and A549 cell lines was significantly higher than that in the HBEC3 and HBEC4 cell lines.

Abbreviations: GPR, G protein-coupled receptor 78.

The team fixed the migrating cells with cold 4% paraformaldehyde, stained them with 1% crystal violet, and quantified the migrated cells using an inverted microscope at 10× magnification (Olympus, Tokyo, Japan) by manual counting.

For cell-invasion detection, the team measured the gap of the wound using a micrograph at a certain time. The team inoculated the cells in the upper compartment of a matrix, glue-coated, polycarbonate membrane filter containing a modified Boyden chamber and the cells in the lower compartment with complete medium and then allowed them to migrate for 24 hours. The team wiped clean the upper surface of the cell membrane, fixed the lower surface with 4% paraformaldehyde, and dyed it with 0.1% crystal violet staining solution.

Cell viability test. The research team used a CCK-8 to measure cell viability, according to the manufacturer's instructions. Before starting the treatment, the team inoculated the cells in a 96-well plate at a density of 5 × 103 cells/well for 24 hours and them incubated the cells with cisplatin at 1 μM, 3 μM, and 10 μM, at 37 °C for 48 hours. The team treated the mixture with the CCK-8 reagent, incubated it at 37 °C for approximately 0.5-3 hours, and determined cell viability by measuring absorbance at 450 nm in a microplate reader. The team used the Prism 7.0 software to calculate the value of the median inhibitory concentration (IC50). The team repeated each experiment three times.

Intervention

To study the cisplatin-induced drug resistance and autophagy of A549, the team incubated 3μM of cisplatin with cells from the control and shRNA-GPR78 groups for 24 hours and collected the cell cultures for detection.

Outcome Measures

Expression of GPR78. The research team analyzed the mRNA expression of GPR78 in the NSCLC cell lines H1975, H1299, and A549 and in the normal human lung epithelial cell lines HBEC3 and HBEC4, using RT-PCR.

A549 cell proliferation. The research team measured the proliferation of the A549 cells using an MTT assay at 0h, 24h, 48h, 72h, and 96h.

Migration and invasion of A549 cells. The research team analyzed the migration and invasion ability of cells using scratch and Transwell tests.

Drug sensitivity of A549 cells. The research team used CCK-8 to detect the cell viability induced by cisplatin at the concentrations 1μM, 3μM, and 10μM.

Arachidonic acid (AA)-induced autophagy. The research team analyzed the protein expression of the autophagy-related factors Beclin-1, LC3-I/LC3-II, p62, and JNK using a Western blot test. The test used human glyceraldehyde-3-phosphate dehydrogenase (GADPH) as the control.

Apoptotic protein. The research team analyzed the protein expressions of caspase-9, caspase-3, and caspase-12 related to apoptosis using a Western blot. The test used human glyceraldehyde-3-phosphate dehydrogenase (GADPH) as the control.

RhoA and Rac1 activities in A549 cells. The research team analyzed the activity of RhoA and Rac1 protein in A549 cells using a Western blot. The test used human glyceraldehyde-3-phosphate dehydrogenase (GADPH) as the control. The Rho family GTP enzymes, including RhoA, Rac1, and Cdc42, are a small (~21kDa) signal G protein family. They are regulators of actin cytoskeleton rearrangement, thus regulating cell migration. For example, RhoA regulates the formation of stress fibers, while Rac1 regulates the actin silk net around cells to produce flaky pseudopodia and membrane folds. It is found that RhoA and Rac1 regulate the elongation of cell morphology and the migration of cancer cells.

Statistical Analysis

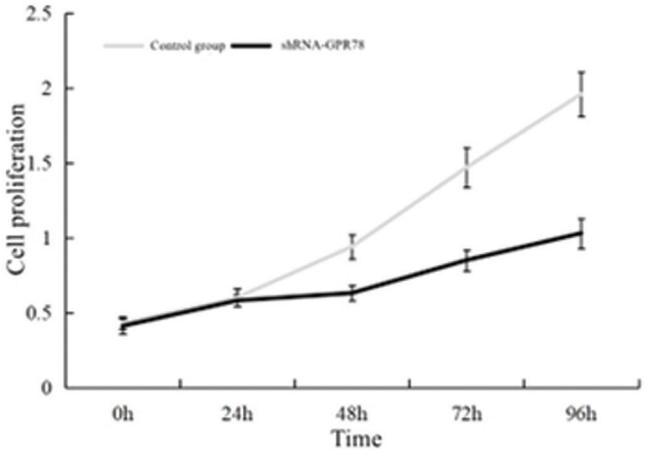
The research team used the SPSS software program, version 20.0 (IBM Corporation, Armonk, New York, USA) for analysis of data. The team expressed the results as means ± standard deviations (SDs), which represented the average at least three independent tests. The team used the double-sample *t* test to determine the statistical difference between two columns' average values. $P < .05$ was considered to be statistically significant.

RESULTS

Expression of GPR78

The mRNA expression of GPR78 in the H1975, H1299, and A549 cell lines was significantly higher than that in the HBEC3 and HBEC4 cell lines ($P < .05$), which indicates that the mRNA level of GPR78 had increased in NSCLC (Figure 1).

Figure 2. Cell Proliferation Curve



*$P < .05$ indicates that the proliferation of A549 cells at 48h, 72h, and 96h in the shRNA-GPR78 group was significantly lower than that of the control group

Abbreviations: GPR, G protein-coupled receptor 78.

A549 Cell Proliferation

At 0h and 24h, no differences existed between the two groups ($P > .05$). At 48h, 72h, and 96h, the proliferation of A549 cells in the shRNA-GPR78 group was significantly lower than that of the control group ($P < .05$). This showed that GPR78 can promote the proliferation of A549 cells (Figure 2).

Migration and Invasion of A549 Cells

The migration and invasion of cells in the shRNA-GPR78 group was significantly lower than those of the control group ($P < .05$), and the decrease in GPR78 expression inhibited the migration and invasion ability of the NSCLC cells (Figure 3 and Table 1).

Drug Sensitivity of A549 Cells

The cell viability of shRNA-GPR78 group was significantly lower than that of the control group ($P < .05$). The data showed that inhibiting the expression of GPR78 could improve the drug sensitivity of NSCLC cells to chemotherapeutic drugs (Table 2).

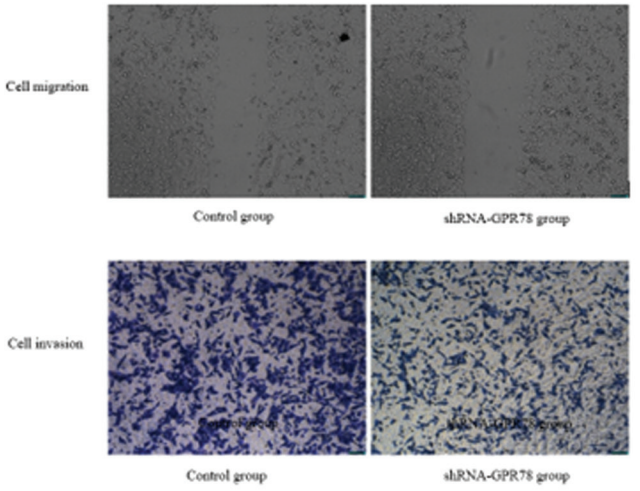
AA-induced Autophagy

Figure 4 and Table 3 show that the protein expression of Beclin-1 and JNK in shRNA-GPR78 group was significantly higher than that of the control group ($P < .05$), while that of the LC3-I/LC3-II and p62 in the shRNA-GPR78 group was significantly lower than that of the control group ($P < .05$).

Apoptotic Protein

The protein expressions of caspase-9, caspase-3, and caspase-12 in the shRNA-GPR78 group were significantly higher than those of the control group ($P < .05$), and knocking down the expression of GPR78 increased the apoptosis of NSCLC cells induced by cisplatin. (Figure 5, Table 4)

Figure 3. Scratch and Transwell Test



Abbreviations: GPR, G protein-coupled receptor 78

Table 1. Detection of Cell Migration and Invasion (n = 12)

Groups	Cell Migration	Cell Invasion
Control group	174.36 ± 32.58	111.57 ± 25.40
shRNA-GPR78 group	72.63 ± 14.27	52.81 ± 10.39
t value	9.908	7.417
P value	<.001[a]	.008[a]

[a]$P < .05$, indicating that the migration and invasion of cells in the shRNA-GPR78 group was significantly lower than those of the control group.

Abbreviations: GPR, G protein-coupled receptor 78.

Table 2. Cell Viability Tests (n =12)

Groups	1μM	3μM	10μM
Control group	83.26 ± 7.42	65.68 ± 10.24	52.18 ± 6.41
shRNA-GPR78 group	65.73 ± 9.33	47.32 ± 6.22	27.33 ± 4.19
t value	5.094	5.308	11.241
P value	<.001[a]	<.001[a]	<.001[a]

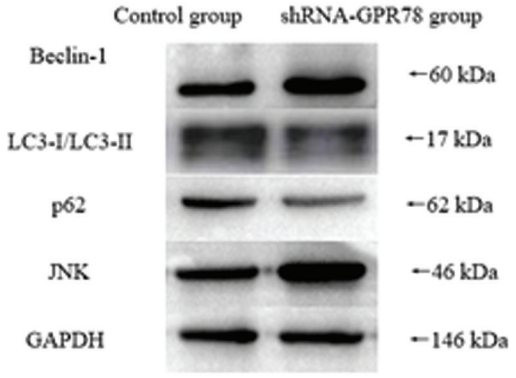
[a]$P < .05$, indicating that the cell viability in the shRNA-GPR78 group was significantly lower than that of the control group.

Abbreviations: GPR, G protein-coupled receptor 78.

RhoA and Rac1 activities in A549 cells

The activity of RhoA and Rac1 protein in the shRNA-GPR78 group was significantly lower than that of the control group ($P < .05$), indicating that the GPR78 regulated the expression of RhoA and Rac1 in A549 cells (Figure 5 and Table 5).

Figure 4. The Expression Levels of Beclin-1, LC3-I/LC3-II, p62, and JNK



Abbreviations: GADPH, glyceraldehyde-3-phosphate dehydrogenase (human); GPR, G protein-coupled receptor 78; JNK, c-Jun N-terminal kinase; LC3-I/LC3-II, microtubule-associated protein light chain 3-I and -II; p62, ubiquitin-binding protein.

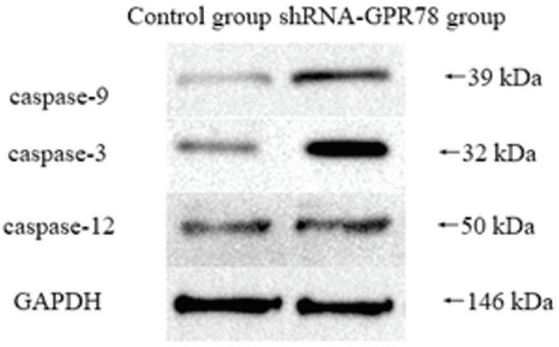
Table 3. The Expression Levels of Beclin-1, LC3-I/LC3-II, p62, and JNK (n =12)

Groups	Beclin-1	LC3-I/LC3-II	p62	JNK
Control group	1.06 ± 0.08	1.95 ± 0.15	1.93 ± 0.12	1.05 ± 0.19
shRNA-GPR78 group	1.92 ± 0.14	1.03 ± 0.02	1.05 ± 0.16	1.92 ± 0.14
t value	-18.476	21.060	15.242	-12.770
P value	<.001[a]	<.001[a]	<.001[a]	<.001[a]

[a]$P < .05$, indicating that the protein expression of the Beclin-1 and JNK was significantly higher and that of the LC3-I/LC3-II and p62 was significantly lower in the shRNA-GPR78 group than the protein expression in the control group.

Abbreviations: GPR, G protein-coupled receptor 78; JNK, c-Jun N-terminal kinase; LC3-I/LC3-II, microtubule-associated protein light chain 3-I and -II; p62, ubiquitin-binding protein.

Figure 5. The Expression Levels of Caspase-9, Caspase-3, and Caspase-12



Abbreviations: GADPH, glyceraldehyde-3-phosphate dehydrogenase (human); GPR, G protein-coupled receptor 78

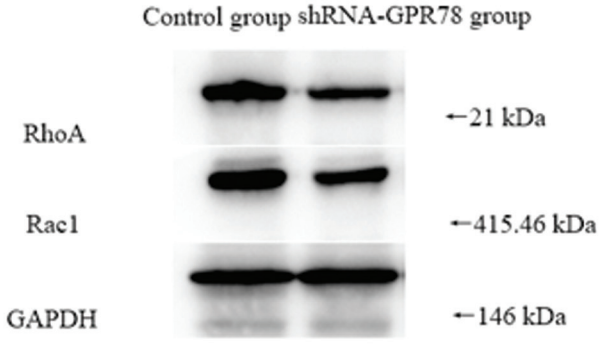
Table 4. The Expression Levels of Caspase-9, Caspase-3, and Caspase-12 (n = 12)

Groups	Caspase-9	Caspase-3	Caspase-12
Control group	1.04 ± 0.13	1.03 ± 0.17	1.06 ± 0.20
shRNA-GPR78 group	1.95 ± 0.16	2.01 ± 0.19	1.95 ± 0.25
t value	-15.291	-13.316	-9.630
P value	<.001[a]	<.001[a]	<.001[a]

[a]$P < .05$, indicating that the protein expressions of caspase-9, caspase-3, and caspase-12 in the shRNA-GPR78 group was significantly higher than that of the control group.

Abbreviations: GPR, G protein-coupled receptor 78.

Figure 6. Western Blot Analysis of Rhoa and Rac1



Abbreviations: GADPH, glyceraldehyde-3-phosphate dehydrogenase (human); GPR, G protein-coupled receptor 78; Rac1, Ras-related C3 botulinum toxin substrate 1; RhoA, Ras homolog family member A

Table 5. Expression Level of Rhoa and Rac1 Proteins (n = 12)

Groups	RhoA	Rac1
Control group	1.95 ± 0.27	1.89 ± 0.15
shRNA-GPR78 group	1.06 ± 0.06	1.03 ± 0.02
t value	11.147	19.687
P value	<.001[a]	<.001[a]

[a]$P < .05$, indicating that the protein expressions of RhoA and Rac1 in the shRNA-GPR78 group was significantly lower than that of the control group.

Abbreviations: GPR, G protein-coupled receptor 78; Rac1, Ras-related C3 botulinum toxin substrate 1; RhoA, Ras homolog family member A.

DISCUSSION

The current study found that knocking down the expression of GRP78 could increase the drug sensitivity of NSCLC cells to cisplatin, which is consistent with previous reports. The current study also found that the various human lung cancer cell lines expressed GPR78, but the normal bronchial epithelial cells didn't. The research team was able to knock down the expression of GPR78 in the NSCLC cell line A549, enabling them to study the cellular function of GPR78. Interestingly, knockdown of GPR78 inhibited cell proliferation, migration and invasion, and increased the drug sensitivity of cisplatin.

The current study examined the molecular mechanism of GPR78 in regulating cell migration and invasion and found that the knockdown of GPR78 inhibited the activities of RhoA and Rac1. This shows that RhoA and Rac1 are responsible for cell migration regulated by GPR78. In addition, the study found that knocking down GPR78 can induce inactivation of RhoA and Rac1 proteins, which may reduce the migration and invasion ability of NSCLC cells.

The current study also found that downregulation of GPR78 can promote the activation of caspase-9, caspase-3, and caspase-12 to promote cisplatin-induced, NSCLC cell apoptosis. That knockdown of GPR78 expression could make cisplatin-resistant NSCLC cells sensitive to cisplatin-induced cell death by regulating the nonclassical cell-death pathway. In the process of autophagy-induced cell death, autophagy may cooperate with the apoptosis signal and induce cell death. The current study showed that GPR78 can inhibit cisplatin-induced autophagy of NSCLC cells, which can contribute to the proliferation, migration, and invasion of NSCLC cells mediated by GPR78.

The current study lacks a complete interpretation of the upstream and downstream targets of the GPR78 signal pathway.

CONCLUSIONS

The current study showed that NSCLC upregulated GPR78. In addition, knocking down GPR78 can weaken the proliferation, migration, and invasion of NSCLC cells, and at the same time, increase cisplatin-induced apoptosis and autophagy of NSCLC cells. Therefore, targeting GPR78 may be a promising treatment strategy for NSCLC patients. The current research team will continue to improve the protocols in subsequent studies.

AUTHORS' DISCLOSURE STATEMENT

This study was supported by Hainan Provincial Natural Science Foundation of China (No. 821MS0822). The authors declare there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Xuqiang Liao and Renzhong Cai contributed equally to this paper. Dr Gao Li is the lead contact.

REFERENCES

1. Dai CH, Shu Y, Chen P, et al. YM155 sensitizes non-small cell lung cancer cells to EGFR-tyrosine kinase inhibitors through the mechanism of autophagy induction. *Biochim Biophys Acta Mol Basis Dis*. 2018;1864(12):3786-3798. doi:10.1016/j.bbadis.2018.10.015
2. Han Q, Rong X, Wang E, Liu S. WW and C2 domain-containing protein-3 promoted EBSS-induced apoptosis through inhibiting autophagy in non-small cell lung cancer cells. *J Thorac Dis*. 2020;12(8):4205-4215. doi:10.21037/jtd-20-966
3. SenthilKumar G, Fisher MM, Skiba JH, et al. FGFR Inhibition Enhances Sensitivity to Radiation in Non-Small Cell Lung Cancer. *Mol Cancer Ther*. 2020;19(6):1255-1265. doi:10.1158/1535-7163.MCT-19-0931
4. Ma S, Miao H, Luo Y, et al. FePt/GO nanosheets suppress proliferation, enhance radiosensitization and induce autophagy of human non-small cell lung cancer cells. *Int J Biol Sci*. 2019;15(5):999-1009. doi:10.7150/ijbs.29805
5. Yin C, Zhang H, Liu X, et al. Downregulated MCOLN1 attenuates the progression of non-small cell lung cancer by inhibiting lysosome-autophagy. *Cancer Manag Res*. 2019;11:8607-8617. doi:10.2147/CMAR.S216538
6. Kim HI, Hong SH, Ku JM, et al. Scutellaria radix promotes apoptosis in non-small cell lung cancer cells via induction of AMPK-dependent autophagy. *Am J Chin Med*. 2019;47(3):691-705. doi:10.1142/S0192415X19500368
7. Wang J, Li J, Cao N, Li Z, Han J, Li L. Resveratrol, an activator of SIRT1, induces protective autophagy in non-small-cell lung cancer via inhibiting Akt/mTOR and activating p38-MAPK. *OncoTargets Ther*. 2018;11:7777-7786. doi:10.2147/OTT.S159095
8. Li F, Li Z, Jin X, et al. Ultra-small gadolinium oxide nanocrystal sensitization of non-small-cell lung cancer cells toward X-ray irradiation by promoting cytostatic autophagy. *Int J Nanomedicine*. 2019;14(8):2415-2431. doi:10.2147/IJN.S193676
9. Han T, Guo M, Gan M, Yu B, Tian X, Wang JB. TRIM59 regulates autophagy through modulating both the transcription and the ubiquitination of BECN1. *Autophagy*. 2018;14(12):2035-2048. doi:10.1080/15548627.2018.1491493
10. Wu X, Xue X, Wang L, et al. Suppressing autophagy enhances disulfiram/copper-induced apoptosis in non-small cell lung cancer. *Eur J Pharmacol*. 2018;827(5):1-12. doi:10.1016/j.ejphar.2018.02.039
11. Lee TG, Kim SY, Kim HR, Kim H, Kim CH. Radiation induces autophagy via histone H4 lysine 20 trimethylation in non-small cell lung cancer cells. *Anticancer Res*. 2020;40(5):2537-2548. doi:10.21873/anticanres.14224
12. Ferrara F, Staquicini DI, Driessen WHP, et al. Targeted molecular-genetic imaging and ligand-directed therapy in aggressive variant prostate cancer. *Proc Natl Acad Sci USA*. 2016;113(45):12786-12791. doi:10.1073/pnas.1615400113
13. Xi J, Chen Y, Huang S, Cui F, Wang X. Suppression of GRP78 sensitizes human colorectal cancer cells to oxaliplatin by downregulation of CD24. *Oncol Lett*. 2018;15(6):9861-9867. doi:10.3892/ol.2018.8549
14. Yeh PS, Wang W, Chang YA, Lin CJ, Wang JJ, Chen RM. Honokiol induces autophagy of neuroblastoma cells through activating the PI3K/Akt/mTOR and endoplasmic reticular stress/ERK1/2 signaling pathways and suppressing cell migration. *Cancer Lett*. 2016;370(1):66-77. doi:10.1016/j.canlet.2015.08.030
15. Dong DD, Zhou H, Li G. GPR78 promotes lung cancer cell migration and metastasis by activation of Gαq-Rho GTPase pathway. *BMB Rep*. 2016;49(11):623-628. doi:10.5483/BMBRep.2016.49.11.133
16. Lin XJ, Liu H, Li P, et al. MiR-936 suppresses cell proliferation, invasion, and drug resistance of laryngeal squamous cell carcinoma and targets GPR78. *Front Oncol*. 2020;10:60. doi:10.3389/fonc.2020.00060
17. Xiong H, Xiao H, Luo C, et al. GRP78 activates the Wnt/HOXB9 pathway to promote invasion and metastasis of hepatocellular carcinoma by chaperoning LRP6. *Exp Cell Res*. 2019;383(1):111493. doi:10.1016/j.yexcr.2019.07.006
18. Liu F, Yang W, Hu M, et al. Constitutive activity of GPR26 regulated by ubiquitin-dependent degradation and its antitumor role. *FEBS J*. 2021;288(15):4655-4682. doi:10.1111/febs.15763
19. Bai Y, Liu X, Qi X, et al. PDIA6 modulates apoptosis and autophagy of non-small cell lung cancer cells via the MAP4K1/JNK signaling pathway. *EBioMedicine*. 2019;42:311-325. doi:10.1016/j.ebiom.2019.03.045
20. Kang SH, Bak DH, Chung BY, Bai HW, Kang BS. Delphinidin enhances radio-therapeutic effects *via* autophagy induction and JNK/MAPK pathway activation in non-small cell lung cancer. *Korean J Physiol Pharmacol*. 2020;24(5):413-422. doi:10.4196/kjpp.2020.24.5.413