GRP78 promotes metastasis by regulation of the endoplasmic reticulum stress through remodeling extracellular matrix in thyroid carcinoma

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Abstract

Background

Thyroid cancer (TC) is the most common type of endocrine malignant tumor and the incidence is increasing by years. Conventional surgery, radiotherapy and chemotherapy are difficult to improve significantly due to the aggression and metastasis of poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid cancer (ATC) which are the most malignant type of thyroid cancer. Glucose-regulated protein (GRP78) as the key molecule is related to tumor growth, apoptosis and metastasis. However, the mechanisms responsible for the effects of TC on GRP78 still need to be discussed. Therefore, the purpose of this study was to explore the presence of GRP78 and the potential mechanism of TC.

Results

GRP78 expression is increased in thyroid carcinoma tissues in comparison with the adjacent normal tissues. Besides, down-regulation of GRP78 significantly inhibited the metastatic and proliferative potential of ATC cells in vitro studies. In addition, tunicomycin (TM)-induced ER stress could up-regulate the expression of GRP78, PERK and XBP1 as well as reverse metastatic ability of GRP78 in TC cells. Bioinformatics and statistical analysis of gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for RNA-seq data from si-GRP78 and si-control showed GRP78 may regulate the ability of metastasis through the ECM remodeling in ATC cells, as well as the expression of ECM components such as COL1A1 and MMP13 were illustrated to be highly relevant to ATC. The analysis of GEPIA database confirmed that high genomic amplification of MMP13 and COL1A1 in TC tissues and were correlated with TNM stage. A further western blot analysis showed MMP13 may be the target of GRP78 in ATC cells and ER stress could activate the expression of MMP13 which was suppressed by depletion of GRP78.

Conclusions

GRP78 is an important regulator of metastasis under the ER stress. In addition, GRP78's functions might be mediated by ECM remodeling in ATC cells, which implicates GRP78 as a therapeutic target in thyroid cancer.

Background

Thyroid cancer (TC) is the most frequent malignancy of the endocrine system and the global incidence rate of which has been increased by 20% over the last 20 years. The global rise in incidence is probably related to differences in diagnostic practices which is considered the main reason. In addition, environmental exposures such as iodine levels and individual risk factors such as obesity are also
contribute to this incidence[1]. Based on the degree of differentiation, thyroid categorized could be divided to three kinds which including well differentiated thyroid carcinomas such as papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), as well as poorly-differentiated thyroid carcinoma (PDTC) and anaplastic thyroid cancer (ATC). Of these forms, although PDTC and ATC accounts for only 15% and less than 5% of thyroid cancer[2], it is responsible for more than half of all thyroid cancer mortalities due to early lymph node (LN) metastasis and invasion of neighboring organs[3]. Standard treatments such as surgery, radiotherapy and chemotherapy has not been successful in treating patients with advanced PDTC and ATC. Therefore, there is an urgent need for a better understanding of the molecular mechanisms underlying TC pathogenesis. The development of TC is believed to be a complex process involving multiple genetic alterations that lead to activation of numerous oncogenic genes and several major signaling pathways such as the p53 mutation and the PI3K/Akt/mTOR pathway[4–6]. Several lines of evidence suggest the potential roles of BRAF, RAS, MMP2, MMP9 and TWIST, in regulating the metastatic process of thyroid cancer[7–11]. In addition, endoplasmic reticulum (ER) stress is believed to contributes to multiple steps along the metastasis and proliferation process in cancers. For example, ER proteins such as XBP1, PERK, ATF6 and ATF4 involved in ER stress have been reported to be participated in tumor growth and metastasis[12–15]. However, the underlying mechanisms responsible for the metastasis and proliferation of thyroid cancer remain poorly understood. In general, new strategy should be exploited to identify novel potential therapeutic targets for patients with TC.

Recent molecular pathological studies have indicated that glucose-regulated protein (GRP78) is involved in tumor development and progression[16–21]. GRP78 (also known as HSPA5) has been demonstrated induced ER stress and overexpression of GRP78 has been found in many human cancers, such as hepatocellular carcinoma, esophagus cancer, gastric cancer and prostate cancer. Besides, several studies have also shown that the expression of GRP78 is related to invasion and metastasis of human cancers. Overexpression of GRP78 is related to increased LN metastasis and poor prognosis in patients with gastric cancer[17], and knockdown of GRP78 has been found to decrease the invasion and ECM degradation in hepatocellular carcinoma cells[19]. These findings demonstrate that GRP78 may take part in the tumor metastasis. However, the role of GRP78 in thyroid cancer has not been clearly elucidated.

In the present study, we found that GRP78 was overexpressed in PTC tissues compared with the peri-carcinoma tissues. Besides, the potential for metastasis and proliferation was decreased in vitro when GRP78 was down-regulated by transwell and CCK8 assays in two ATC cell lines, ARO and FRO cells. Moreover, tunicamycin as a ER stress inducer could up-regulate the expression of GRP78 and related molecules such as XBP1s and PERK, as well as promote the metastasis in ATC cells. Furthermore, downregulation the expression of GRP78 could reverse the effect of tunicamycin at least in part, which suggested that deletion of GRP78 could suppresses tunicamycin-mediated ER stress and metastasis in ARO and FRO cells. To further clarify the specific mechanism of GRP78 in TC, RNA sequencing analysis (RNA-seq) was performed to explore the metastasis-related genes in ARO and FRO cells. Here, we analyzed RNA-sequencing (RNA-seq) data from si-GRP78 and si-control to identify differentially expressed genes and pathways according to GO enrichment and
KEGG pathways in both cells, the result showed that GRP78 may regulate the ability of metastasis by remodeling synthesis of extracellular matrix in ARO and FRO cells. Among these genes, ECM components such as COL1A1 and MMP13 are obviously related to GRP78, As COL1A1 and MMP13 have been reported to be related with metastasis of many cancers[22–24], to further confirmed this results, GEPIA database was used and confirmed that high expression of MMP13 and COL1A1 in TC tissues and were correlated with TNM stage. Moreover, depletion of GRP78 can result in a significant reduction of MMP13 and ER stress could reverse the decline. Of course, the outcomes remain to be elucidated in more detail.

reversed the increase

Results

High expression levels of GRP78 in thyroid carcinoma

GRP78 protein expression levels in thyroid carcinoma and peri-carcinoma tissues were examined by immunohistochemistry(IHC) assay. As shown in Fig. 1a, GRP78 immunoreactivities were observed in both the cytoplasm and membranes of the tissues. The positive staining score of GRP78 in carcinoma tissues is obvious higher than peri-carcinoma tissues(Fig. 1b). However, GRP78 expression had no significant correlations with different stage(Fig. 1c). Due to the aggressiveness and high mortality of ATC, two undifferentiated human thyroid carcinoma cell lines, ARO and AFO were selected for the following test. Taken together, these findings suggest that the expression of GRP78 is higher in thyroid carcinoma.

GRP78 Promotes Metastasis And Proliferation Of Thyroid Cancer Cells

To elucidate whether the roles of GRP78 is involved in TC cells, we set out to perform in vitro assays to assess whether GRP78 enhances the proliferation and migration of TC cells, GRP78 siRNA was transfected into ARO and FRO cells. The downregulation of GRP78 was confirmed by western blot analysis(Fig. 2a). The results of transwell assays showed that the migratory ability of GRP78 siRNA-transfected cells was significantly reduced compared with control cells(Fig. 2c). In addition, CCK-8 assay was used to investigate the effects of GRP78 on proliferation inhibit, and the result also confirmed that proliferation of GRP78 siRNA-transfected cells was markly inhibited compared with control ones in ARO cells(Fig. 2b). Taken together, these results indicated that down-regulation of GRP78 could significantly inhibit ARO and FRO cells migration and proliferation of ARO cells in vitro.

Suppression of GRP78 expression partially decreases ER stress agonist-induced metastasis in thyroid cancer cells

Since we found that the GRP78 expression levels are correlated with metastasis in ARO and FRO cells. As ER stress exerts role that contributes to metastasis on cancer cells. To understand the molecular mechanism underlying the GRP78-mediated suppression of metastasis in thyroid cancer, we set out to
perform in vitro assays to determine whether the GRP78 protein expression levels and ability of metastasis are upregulated under the ER stress condition. ARO and FRO cells were treated with different doses of tunicamycin (TM) (0–1 µg/mL). The results indicated that TM(1 µg/mL) could trigger ER stress and elevate expression of GRP78, PERK and XBP1 by using Western blotting in both cells (Fig. 3a), as well as TM-induced ER stress could enhance the ability of metastasis by using transwell assays in both cells (Fig. 3b). In addition, we found that the downregulated GRP78 protein expression levels and the ability of metastasis were both reversed by TM-induced ER stress (Fig. 3c). These results indicate that ER stress induced upregulation of GRP78 is involved in enhancing the migration of thyroid cancer cells.

**Depletion of GRP78 altered the expression of ECM related molecules and ECM remodeling pathway**

The results described above have demonstrated the GRP78 could regulate metastasis by increase the level of ER stress in ATC cells. To further delineate the mechanisms underlying the GRP78 in ATC cells, we collected the GRP78 and si-GRP78 for RNA-sequencing in ARO and FRO cells. The results showed that 119 genes were upregulated and 38 genes were down regulated in ARO cells (Fig. 4a), while 48 genes were upregulated and 26 genes were down regulated in ARO cells (Fig. 4b). Furthermore, GO and The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed to explore the functional roles of differentially expressed genes (DEGs) in ATC cells. The GO analysis was performed and showed the location terms that were significantly over-represented in this set were proteinaceous extracellular matrix, extracellular matrix component and basement membrane. In the GO analysis of molecular function and biological processes, one clear conclusion from our RNA-seq analysis is, therefore, GRP78 were mainly related to the regulation of metastasis by ECM remodeling pathway in thyroid cancer cells. Next, KEGG pathway analysis showed enrichment in a series of pathways, such as GnRH signaling pathway, ECM-receptor interaction and protein processing in endoplasmic reticulum, all of which are critical to the process of tumor metastasis. In ECM-receptor interaction pathways, ECM components such as COL4A1, COL1A1 and MMP13 were illustrated to be highly relevant to TC cancer.

**MMP13 is increased in thyroid carcinoma and may be the target of GRP78**

Based on the results of sequencing and bioinformatics analysis, Interestingly, MMP13 and COL1A1 were reported to contributes to occurrence and development of tumor, especially the metastasis of tumor. To further verify the role of MMP13 and COL1A1 in TC, the data contained the difference of MMP13 and COL1A1 expression in 512 tumor tissues and 337 corresponding normal tissues generated by using Gene Expression Profiling Interactive Analysis (GEPIA) database. The result revealed high genomic amplification of MMP13 and COL1A1 in TC tissues (Fig. 5a). Moreover, the genomic amplification of MMP13 and COL1A1 were related to individual cancer stage (Fig. 5b). To further verify these results, western blot was performed to determine whether the protein expression of MMP13 and COL1A1 had the same variation. The results showed depletion of GRP78 result in a significant reduction of MMP13 expression in both cells (Fig. 5c). In addition, ER stress could reverse this variation caused by down-regulation of GRP78 in ARO cells (Fig. 5d). However, there is no significant trend on COL1A1. which further
verified that MMP13 may be the target of GRP78. Of course, this needs to be confirmed by further experiments.

**Discussion**

Thyroid cancer is the most commonly diagnosed endocrine malignancy with an increasing incidence in the past decades worldwide. PTC accounts for 80% of thyroid cancer and have a better prognosis. However, patients with PDTC or ATC, due to rapid progress and local or distant metastasis, frequently tend to have a worse prognosis[1–4]. In addition to the characteristics of rapid growth of tumor cells, metastasis is one of cancer hallmarks and leads tumor dissemination and aggressiveness[25]. In the process, cancer cells becomes polarized in order to cross the surrounding ECM and stromal cell layers, then travel to distant sites and eventually grow into new metastatic colonies through the cardiovascular or lymphatic circulatory systems[26]. Metastatic potential as the main reason of the curative failures in cancers and in cancer-related mortality which involves many molecules and pathways. Our previous research shows that GRP78 is overexpressed and correlated with invasion, metastasis and poor prognosis in ESCC patients[18]. Besides, we also found that the expression of GRP78 was significantly higher in multidrug resistance gastric cancer cells and the knockdown of GRP78 significantly reversed multidrug resistance in gastric cancer[16, 20]. All these studies confirmed that GRP78 is involved in the pathological process of numerous cancers. In our study, immunohistochemistry results of 62 PTC patients reveal that the expression of GRP78 was significantly higher compared with the peri-carcinoma tissue in PTC patients. However, patients with positive GRP78 expression had no significant correlations with degree and differentiation. Thus, to elucidate whether the roles of GRP78 is involved in thyroid cancer cells, transwell and CCK8 arrays was applied and the results indicated that down-regulation of GRP78 could significantly inhibit ATC cells migration and proliferation in vitro. Thus, our studies suggested that GRP78 promote TC cells growth and migration.

The endoplasmic reticulum (ER) as a significant component of the endomembrane system that modifies dysfunctional proteins and prevents them from secretion in eukaryotic cells. All the factors that affect the function of endoplasmic reticulum can cause endoplasmic reticulum stress, several adverse conditions, such as glucose deprivation, acidosis, and severe hypoxia, could trigger ER stress and lead to the accumulation of misfolded proteins and production of unfolded protein response (UPR) in ER. Growing evidence suggests that the ER stress is not only a physiological state but also actively contributes to occurrence and development of tumor. Upon ER stress, GRP78, also referred to as BiP (immunoglobulin heavy-chain binding protein), serves as an ER stress signaling regulator and plays critical roles in the stress of oncogenesis. PERK and XBP1 as the most important ER membrane proteins have been recently linked to tumor cell migration/invasion processes such as ECM and EMT[27–29]. For example, Overexpression of XBP1 could promote cell invasion and metastasis through the upregulation of MMP9 in esophageal squamous cell carcinomas[27]. Similarly, PERK is also found to contribute to ECM reorganization in breast cancer and overexpression of ATF4 which is a component of PERK pathway induce cell invasion and metastasis stimulating MMP2 and MMP7 expression in esophageal squamous cell carcinomas[13]. These evidences show that UPR activation might be relevant for the development of
tumor metastasis. To now, there is several ER stress inhibitors such as ATF6 inhibitor were reported to display a strong effect on inhibiting tumor migration of the large range of cancer types including brain, breast, liver, lung, pancreas and skin. As part of UPR, the protein GRP78 could regulate multiple signaling pathways associated with metastasis, drug resistance, and immune function. In order to characterize the effect of ER stress on thyroid cancer, tunicomycin(TM) as the ER inducer was used and the result showed TM can not only up-regulate the expression of GRP78, PERK and XBP1, but also promote the ability of cell metastasis in ARO and FRO cells. Besides, downregulation of GRP78 could reverse metastatic ability and TM-induced ER stress in ATC cells. The findings indicated that GRP78 as the major molecular chaperone in the endoplasmic reticulum which could promote metastasis by increasing the level of endoplasmic reticulum stress in ARO and FRO cells.

The extracellular matrix (ECM) is a complex dynamic structure that is present in all tissues and continuously undergoes degradation and remodeling. In addition to the provision of structural support for cells, the ECM can interact with cells through cell surface ECM receptors or other ways to regulate cell functions, such as proliferation, migration and differentiation[30]. Abnormal ECM remodeling and degradation could influence cell fate and behavior, which result some several pathological conditions, such as fibrosis and invasive cancer[31]. Matrix metalloproteinases (MMP) as extracellular or membrane-bound enzymes play critical roles in ECM remodeling[32]. A better understanding of how ECM remodeling affects disease progression will contribute to the development of new therapeutics. In our study, GO and KEGG enrichment analysis were applied to screened the differentially expressed genes and the pathways in ATC cells, these results found that GRP78 may regulate the ability of metastasis through the ECM remodeling pathway in TC cells. Among these genes, ECM components such as COL1A1(alpha-1 type I collagen) and MMP13 are obviously related to the ECM remodeling pathway. Previous investigations indicated that COL1A1 and MMP13 are associated with cancer metastasis[22–24, 33]. In order to investigate the effects of COL1A1 and MMP13 on TC, the data contained the difference expression of MMP13 and COL1A1 in 501 tumour tissues and 58 adjacent normal tissues generated by using GEPIA datasets(Gene Expression Profiling Interactive Analysis)[34]. the result revealed high genomic amplification of MMP13 and COL1A1 in TC tissues and overexpression of MMP13 and COL1A1 are related to advanced TNM stage. Besides, ER stress could activate the expression of MMP13 which was suppressed by depletion of GRP78. Although more accurate experiments are required to verify these results.

Conclusions

In summary, the results revealed the mechanism of GRP78 mediated ER stress-metastasis in ATC cells is associated with the ECM remodeling pathway. Effective overexpression of GRP78 remarkably ER stress-metastasis. Of note, we also show for the first time in our knowledge that GRP78 may promotes metastasis via regulating the expression of MMP13. Better understanding of the mechanism will allow to more specifically target the relevant actors to prevent tumor metastasis and improve a novel therapeutic target for patients with thyroid carcinoma.
Methods

Cell culture and TC tissues

The undifferentiated ATC cell lines ARO and FRO were initially obtained from the Chinese Academy of Medical Science. All cell lines were maintained in RPMI 1640 (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (ZETA, USA) at 37 °C in a humidified air atmosphere containing 5% CO2. The cells were used in the logarithmic phase of growth throughout the experiment. The TC tissue microarrays were obtained from ShGnghGi Outdo Biotech CompGny (Shanghai, China). Each array contained TC tissues and adjacent TC tissues from a total of 62 cases.

Immunohistochemical Analysis (IHC) And Pathology Scores

As described previously[18], IHC staining was performed according to the manufacturer’s instructions. The tissue microarrays were incubated at 4°C overnight with anti-GRP78 polyclonal antibody (1:100, Santa Cruz, CA, USA). The immunoreactivity proportion were classified as follows (percentage scores): < 5% (0), 5–25% (1), 25–50% (2), 50–75% (3), and > 75% (4). The staining intensities were classified as follows (intensity scores): negative (0), weak (1), moderate (2), and strong (3). The final score was obtained by percentage scores × intensity scores. Total scores ranging from 0 to 4 were defined as low group, and total scores ranging from 5 to 12 were defined as the high group.

Lentivirus-mediated siRNA Construction And Cell Transfection

The lentivirus-mediated small interfering RNA for GRP78 (siGRP78) and the negative control RNA (siNC) were designed and synthesized by Genepharma (Shanghai, China). The GRP78 siRNA sequence was: F: 5-GGUACUGCUUG

AUGUAUGUTT-3, R: 5- ACAUACAUCAAGCAGUACCTT 3; The control siRNA sequence was: F: 5- UUCUCCGAACGUGACACGUTT-3, R: 5-ACGUGACACGUUCCGAGAATT-3. For transfection experiments, ARO and FRO were plated in 6-well plates and siRNAs were transfected using LipofectamineTM 2000 reagent (Invitrogen, USA) according to the manufacturer’s protocol for 24 hours. the efficiency of gene silencing was further confirmed by western blot analysis in both cells.

Tunicamycin(TM) Treatments
ARO and FRO cells were seeded in 6-well plates at $3 \times 10^5$ cells/mL in a total volume of 3 mL. Post 24-hrs of inoculation, different concentrations of TM (0–1 µg/mL) was added and the cells were cultured for another 72 hours until day. A parallel untreated culture with same passage number as adapted cultures was maintained as control cultures, the level of ER-stress was further confirmed by western blot analysis in both cells.

**Western Blot Assay**

The western blot were performed as described previously[20]. The antibodies used for the western blot were PERK, XBP1s and CLO1A1 (Abcam, USA), GRP78 and MMP13 (Santa Cruz, CA, USA), and β-actin (Beyotime Institute of Biotechnology Jiangsu, China).

**Cell Proliferation**

Cell counting kit-8 (CCK-8) (Dojindo laboratories, Kumamoto, Japan) was used to measure cell proliferation. Cells were inoculated in 96-well plate, pre-incubated for 24 h and then incubated for 0 h, 24 h, 48 h, 72 h or 96 h (5% CO2, 37 °C). At which point, 10 µL CCK-8 solution was added to each well and cells were further incubated for one to three hours. The absorbance (OD value) at 450 nm was measured with a microplate reader.

**Transwell Migration Assays**

Transwell chambers were used for migration assays in vitro. as described previously[12], $5 \times 10^4$ cells were seeded per well in the upper chamber (8 µm pore size, Corning, USA). After incubation for 24 h at 37 °C, the wells were washed with PBS three times and then fixed with methanol for 1 h and stained with 1% crystal violet for 30 min. The cells on the upper surface of the filter were scraped off, and the cells on the lower surface of filters were counted by a light microscope (Olympus BX51, Olympus) at 2009 magnification in ten randomly selected fields. The experiment was repeated independently three times.

**GO Enrichment, KEGG Pathway Analysis Of RNA-seq Data**

RNA-Sequencing data of GRP78 and si-GRP78 in ARO and FRO cells were tested by BGI (Shenzhen, China)[35]. The functional roles of differentially expressed genes (DEGs) were revealed by determining the transcriptional profiles acquired using RNA-seq and by GO and KEGG enrichment analyses. We used the GO database to analyze the functional enrichment of DEGs, focusing on the pathways associated with these genes that were enriched in the terms biological process, molecular function and cellular component. The KEGG pathway database was used to determine the enrichment of DEGs.

**GEPIA database for differential expression analysis of MMP13 and CLO1A1 genes**
Differential expression of MMP13 and CLO1A1 genes between 512 TC tissue and 337 normal tissue was acquired from GEPIA (http://gepia.cancer-pku.cn/)[35]. In addition, we further explored the differential expression of MMP13 and CLO1A1 genes in terms of pathological stage(I-IV) was analyzed among the TC patients.

**Statistical analysis**

Statistical significance was calculated with GraphPad Prism Software using one-way ANOVA with Tukey's post-test and Student's t-test. Data are presented as mean ± SD. Differences with $p < 0.05$ were considered statistically significant.

**Abbreviations**

TC: Thyroid cancer; PTC: Papillary thyroid carcinoma; FTC: Follicular thyroid carcinoma; PDTC: Poorly differentiated thyroid cancer; ATC: Anaplastic thyroid cancer; GRP78: Glucose-regulated protein78; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; LN: Lymph node; ER: Endoplasmic reticulum; RNA-seq: RNA sequencing analysis; IHC: Immunohistochemistry; TM: Tunicamycin; DEGs: Differentially expressed genes; UPR: Unfolded protein response; ECM: Extracellular matrix; MMP: Matrix metalloproteinases.

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Ethical Committee of Tai zhou Hospital, and written consent was obtained from all patients.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets generated during and/or analyses during the current study are available in GEPIA database

**Competing interests**

The authors declare that they have no competing interests.

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Authors’ contributions

Guohong Zhao, Jianqin Kang and Guanghui Xu contributed equally to this work.

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Figures
Figure 1

GRP78 expression is increased in thyroid carcinoma. (a) IHC for the expression of GRP78 in TC tissues compared to peri-tumor tissues. (b) The scores of IHC show increased levels of GRP78 in normal tissues compared with peri-tumor tissues. **P < 0.01. (c) The scores of IHC show the levels of GRP78 in stage I-II compared with stage III-IV.
Knockdown of GRP78 suppresses proliferation and metastasis in ARO and FRO cells. (a) Western blot analysis of GRP78 protein expression in ARO and FRO cells transfected with si-NC, si-GRP78-1 and si-GRP78-2. (b) CCK-8 proliferation assays were used to determine the viability of siGRP78-transfected ARO and FRO cells. **P < 0.01. (c) Transwell assays were performed to assess the migration of siGRP78-transfected ARO and FRO cells. Bar graphs represent the average migration rate of the ARO and FRO cells. ***P < 0.001.
Figure 3

Suppression of GRP78 expression partially decreases ER stress agonist-induced metastasis in thyroid cancer cells. (a) Western blot analysis of XBP1s, PERK and GRP78 protein expression in ARO and FRO cells adding with TU(0-1 μg/L). (b) Transwell assays were performed to assess the migration of ARO and FRO cells adding with TU(1 μg/L). Bar graphs represent the average migration rate of the ARO and AFO cells, ***P < 0.001, ****P < 0.0001. (c) Transwell assays were performed to assess the migration of siGRP78-transfected ARO and FRO cells adding with TU(1 μg/L). Bar graphs represent the average migration rate of the ARO and AFO cells, *P < 0.05, ***P < 0.001, ****P < 0.0001.
Depletion of GRP78 altered the expression of ECM related molecules and ECM remodeling pathway. (a) Volcano plot of differentially expressed mRNAs between normal si-NC and si-GRP78 of ARO and FRO cells. (b) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis of DEGs of ARO and FRO cells. (c) GO enrichment terms and KEGG pathway analysis of DEGs of ARO and FRO cells. The statistically significant enriched GO terms in Biological Process, Cellular Component, and Molecular Function. The FDR corrected p values are displayed on a -log10 scale.
Figure 5

Analysis the expression of MMP13 and COL1A1 in ATC cells and MMP13 may be the target of GRP78. (a) Data from GEPIA database show the mRNA expression of COL1A1 and MMP13 in normal tissues and TC tissues. Red boxes represent tumor tissues, and grey boxes represent normal tissues. Based on individual cancer stages. (b) Expression of COL1A1 and MMP13 in different stages of thyroid carcinoma. (c) Western blotting analysis of MMP13 and COL1A1 in GRP78 knockdown cells. (d) Western blotting analysis of MMP13 and COL1A1 in GRP78 knockdown cells adding with TU(1μg/L).