DDIT4 Novel Mutations in Pancreatic Cancer

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Pancreatic cancer is one of the most common malignancies worldwide. This study is aimed at searching the possible genetic mutations and the value of novel gene mutation in the DNA damage-inducible transcript 4 (DDIT4) and signaling pathway in pancreatic cancer. Polymerase chain reaction (PCR) was performed to amplify the DNA sequences of DDIT4 from patients with pancreatic ductal adenocarcinoma. In addition, we used IHC to detect the expression level of DDIT4 in patients with pancreatic cancer in different types of gene mutation. Double-labeled immunofluorescence was employed to explore the expression levels of DDIT4/LC3 and their potential correlation. Our work indicated the two novel stable gene mutations in DDIT4 mRNA 3′-untranslated region (m.990 U>A and m.1246 C>U). Thirteen samples were found to have mutation in the DDIT4 3′-untranslated regions (UTR). To further verify the influence of gene mutation on protein expression, we performed immunohistochemistry on different gene mutation types, and we found a correlation between DDIT4 expression and gene mutation, which is accompanied by nuclear staining deepening. In order to further discuss the clinical value of DDIT4 gene mutation, immunofluorescence suggested that the expression of DDIT4 colocated with LC3; thus, we speculated that DDIT4 mutation may be involved in autophagy in pancreatic cancer cell. In this study, we found mutation in the 3′-UTR region of DDIT4, which may be associated with DDIT4 expression and tumor autophagy in pancreatic cancer tissues.

1. Introduction

Pancreatic cancer ranked the fourth most common cause of fatalities due to cancer in America. The 5-year relative survival rate of patients with pancreatic cancer was only 9% [1]. According to the research of the World Health Organization (WHO), the observed number of deaths due to pancreatic cancer was 82901 in 2014 in Europe [2]. Despite advances in conventional therapies, little improvement has been observed in the survival rate over the past 30 years [3]. Many patients with pancreatic ductal adenocarcinoma (PDAC) are either intrinsically resistant or develop acquired resistance to radiotherapy [4]. SMAD4 mutation rendered pancreatic cancer resistance to radiotherapy through promotion of autophagy [5]. However, the underlying role of gene mutation has not been fully elucidated and the mutation of autophagy-related gene will be a great treasure.

The somatic and germline genetic mutation contributes to the occurrence of pancreatic cancer, and the special gene is a novel target to kill the cancer cell. Wood et al. summarized the 24 sporadic gene mutations including the common gene mutation (KRAS, CDKN2A, TP53, SMAD4, GNAS, and RNF43) which is more than 20% prevalence [6]. Gene mutation involved in the resistance to radiotherapy in patients with pancreatic cancer [5] and the autophagy is associated with the adaptation to harsh microenvironment [7].
| No. | Gender | Stages   | Surgical method                          | Outcome                             | Chemotherapy regimens                                                                 | Pathology                                      | Image features | Tumor markers                                                                 |
|-----|--------|----------|------------------------------------------|-------------------------------------|---------------------------------------------------------------------------------------|------------------------------------------------|----------------|-------------------------------------------------------------------------------|
| 1   | Female | IIB      | Radical pancreaticoduodenectomy         | Adjuvant therapy                    | (Gemcitabine 1.4 g D1, 8, 15 q4w) * 1 cycle                                           | Moderately differentiated adenocarcinoma       | 2.5 * 2.5 * 2 cm | HER2(0), P53(70%+), MUC6(-), MUC2(-), MUC1(+), CDX2(-), MUC5A(+)              |
|     |        | (pT2N1M0)|                                         |                                     |                                                                                       |                                                |                | SATB2(-), CK20(-), CK7(+), CK19(+), CD54(+)                                   |
|     |        |          |                                          |                                     |                                                                                       |                                                |                | β-Catenin(+), vim(+), CK(-), CD10(+), syn(-), EMA(-), CEA(+), CDX2(-), AFP(-), |
|     |        |          |                                          |                                     |                                                                                       |                                                |                | CK(-), CK8/18(-), WT-1(-), MUC1(-), MUCSA(-)                                  |
|     |        |          |                                          |                                     |                                                                                       |                                                |                | P53(+), HER2(1+), Ki-67(80%), MUC1(+), MUC2(-), MUC5(-), MUC6(-)              |
| 2   | Female | IB       | Partial pancreatectomy with duodenal reservation | Adjuvant therapy                    | Unknown                                                                              | Solid pseudopapilloma                           | 3.2 * 3.0 * 1.8 cm | MUC1(+), MUC5(-), MUC6(-), MUC7(-), MUC19(-), MUC156(-), MUC162(-)           |
|     |        | (pT2N0M0)|                                         |                                     |                                                                                       |                                                |                | MUC1(+), MUC2(-), MUC5(+), MUC6(-), CK7(+), CK20(-), CDX2(+), Ki-67(+60%),   |
|     |        |          |                                          |                                     |                                                                                       |                                                |                | P53(+), HER2(1+), Ki-67(80%), MUC1(+), MUC2(-), MUC5(-), MUC6(-)              |
| 3   | Male   | IIA      | Radical pancreaticoduodenectomy         | Liver metastasis                    | (Gemcitabine 0.8 g D1, D8 + cisplatin 30 mgD1-2, q3w) * 2 cycles                      | Moderately differentiated adenocarcinoma       | 2.5 * 2.4 * 2 cm | MUC1(-), MUC5(-), MUC6(-), MUC7(-), MUC19(-), MUC156(-), MUC162(-)           |
|     |        | (T3N0M0) |                                         |                                     |                                                                                       |                                                |                | MUC1(+), MUC2(-), MUC5(+), MUC6(-), CK7(+), CK20(-), CDX2(+), Ki-67(+60%),   |
|     |        |          |                                          |                                     |                                                                                       |                                                |                | P53(+), HER2(1+), Ki-67(80%), MUC1(+), MUC2(-), MUC5(-), MUC6(-)              |
| 4   | Female | IIIA     | Partial pancreatectomy                  | Did not receive chemotherapy because general condition is bad | (Gemcitabine 1.4 g D1, D8 + oxaliplatin 150 mgD1 ) * 1 cycle; (gemcitabine 1.4 mg D1, D8 + tegafur 3#qm, 2#qn D1-D14) * 2 cycles | Moderately differentiated adenocarcinoma       | 3.5 * 3 * 2 cm  | MUC1(+), MUC5(-), MUC6(-), MUC7(-), MUC19(-), MUC156(-), MUC162(-)           |
|     |        | (pT4N0M0)|                                         |                                     |                                                                                       |                                                |                | MUC1(+), MUC2(-), MUC5(-), MUC6(-), CK7(+), CK20(-), CDX2(+), Ki-67(+60%),   |
|     |        |          |                                          |                                     |                                                                                       |                                                |                | P53(+), HER2(1+), Ki-67(80%), MUC1(+), MUC2(-), MUC5(-), MUC6(-)              |
| 5   | Male   | IIIA     | Partial pancreatectomy                  | Septic shock and death               | Vacant                                                                               | Moderately differentiated adenocarcinoma       | 1.5 * 1 * 0.7 cm | MUC1(+), MUC5(-), MUC6(-), MUC7(-), MUC19(-), MUC156(-), MUC162(-)           |
|     |        | (pT4N0M0)|                                         |                                     |                                                                                       |                                                |                | MUC1(+), MUC2(-), MUC5(-), MUC6(-), CK7(+), CK20(-), CDX2(+), Ki-67(+60%),   |
|     |        |          |                                          |                                     |                                                                                       |                                                |                | P53(+), HER2(1+), Ki-67(80%), MUC1(+), MUC2(-), MUC5(-), MUC6(-)              |
| 6   | Male   | III      | Partial pancreatectomy with duodenal reservation | Local recurrence and liver metastasis | (Gemcitabine 1.4 g D1, D8 + oxaliplatin 150 mgD1 ) * 1 cycle; (gemcitabine 1.4 mg D1, D8 + tegafur 3#qm, 2#qn D1-D14) * 2 cycles | Moderately differentiated adenocarcinoma       | 1.5 * 0.8 * 0.4 cm | MUC1(+), MUC2(-), MUC5(-), MUC6(-), CK7(+), CK19(+), EMA(+), CA199(+), Ki-67(10%) |
|     |        | (pT1N2M0)|                                         |                                     |                                                                                       |                                                |                | MUC1(+), MUC2(-), MUC5(-), MUC6(-), CK7(+), CK19(+), EMA(+), CA199(+), Ki-67(10%) |
|     |        |          |                                          |                                     |                                                                                       |                                                |                | MUC1(+), MUC2(-), MUC5(-), MUC6(-), CK7(+), CK19(+), EMA(+), CA199(+), Ki-67(10%) |
| 7   | Male   | IV       | Radical pancreaticoduodenectomy         | Adjuvant therapy                    | (Capecitabine 1.5 g bid D1-14 + gemcitabine 1.4 g D 1, 8, q3w) * 8 cycles             | Moderately differentiated adenocarcinoma       | 5.5 * 4.0 * 3.0 cm | MUC1(+), MUC2(-), MUC5(-), MUC6(-), CK7(+), CK19(+), EMA(+), CA199(+), Ki-67(10%) |
|     |        | (pT2n1m1)|                                         |                                     |                                                                                       |                                                |                | MUC1(+), MUC2(-), MUC5(-), MUC6(-), CK7(+), CK19(+), EMA(+), CA199(+), Ki-67(10%) |

Table 1: Basic clinical characteristics of patients.
| No. | Gender | Stages       | Surgical method               | Outcome            | Chemotherapy regimens                                      | Pathology                                 | Image features | Tumor markers                                      |
|-----|--------|--------------|-------------------------------|--------------------|-------------------------------------------------------------|-------------------------------------------|----------------|---------------------------------------------------|
| 8   | Female | IIB (pT3N1M0)| Radical pancreaticoduodenectomy | Adjuvant therapy   | (Gemcitabine 1.6 g D1, D8 + capecitabine 2 g D2-D15) * 6 cycles mFOLFIRINOX * 4 cycles (Gemcitabine 1.4 g + albumin paclitaxel 200 mg) * 1 cycle | Poorly differentiated adenocarcinoma     | 3 * 3 * 2.5 cm | CK20(+), CK7(+), MUC5(+), MUC1(+), CK818(+), CK19(+), EMA(+), CA199(+), Ki-67(10%), MUC2(-), D2-40(+), CD34(+) |
2.1. Patient Recruitment and Data Collection. Patients with pancreatic neoplasm from the First Affiliated Hospital of Fujian Medical University were included between 2018 and 2019. The patients we included were set as the experimental group and the control group, respectively, according to the pathological results. The experimental group is the group of patients with pancreatic cancer and the control group is the group of patients with paracancerous tissue. The inclusion criteria are as follows. (1) The patients had complete clinical data and follow-up data. (2) The patients signed informed consent. Patients with any comorbid or previous malignancies were excluded from the study. The related information of patients with pancreatic cancer was collected including age, gender, TMN stage, surgical method, outcome, chemotherapy regimen, pathological grading, imaging features, and the level of tumor markers (Table 1). This study was approved by the Ethics and Research Committee of the First Affiliated Hospital of Fujian Medical University.

2.2. Genotype Analysis. Pancreatic neoplasm tissue and adjacent healthy tissue samples were collected in liquid nitrogen and stored at -80°C. A TIANamp tissue DNA extraction kit (Tiangen Biotechnology Co., LTD.) was used to extract and store DNA from pancreatic cancer tissues and control tissues. The full-length DDIT4 was amplified and underwent gel electrophoresis following purification. Multiple sequencing was performed and analyzed by Sangon Biotech Co., Ltd. The forward primer of PCR was 5′-GTTCGCACACC CATTCAA-3′, and the reverse one was 5′-GCATAGGTC TTAATACGTGACAT-3′. The condition of PCR was initial denaturing step, and an ABI PRISM 7700 sequencer (PerkinElmer, Inc.) was used for gene sequencing.

2.3. Immunohistochemistry and HE Staining. Pancreatic tissues from patients with pancreatic cancer were used for hematoxylin and eosin staining and immunohistochemistry. DDIT4 antibodies were obtained from Arigo Biolaboratories (Hsinchu, Taiwan). Eight-micrometer sections were made in all paraffin-embedded pancreatic tissue. The slides were immunolabeled with monoclonal antibodies against DDIT4 (DNA damage-inducible transcript 4). Immunohistochemistry was performed using an automated immunohistochemical stainer according to the manufacturer’s guidelines. Substitution of the primary antibody with phosphate-buffered saline was used as a negative control.

### Table 2: Relationship between DDIT4 mutation and pancreatic cancer.

| N  | Gender | Tissue types | Pathologic types                  | mRNA.990 | mRNA.1246 |
|----|--------|--------------|-----------------------------------|----------|-----------|
| 1  | Female | Cancerous    | Moderately differentiated adenocarcinoma | T>A      | C>T       |
| 2  | Female | Paracancerous| —                                 | T>A      | C>T       |
| 3  | Female | Cancerous    | Solid pseudopapilloma              | N        | N         |
| 4  | Female | Paracancerous| —                                 | N        | N         |
| 5  | Male   | Cancerous    | Moderately differentiated adenocarcinoma | N        | N         |
| 6  | Male   | Paracancerous| —                                 | N        | N         |
| 7  | Female | Cancerous    | Moderately differentiated adenocarcinoma | T>A      | C>T       |
| 8  | Female | Paracancerous| —                                 | T>A      | C>T       |
| 9  | Male   | Cancerous    | Moderately differentiated adenocarcinoma | N        | C>T       |
| 10 | Male   | Paracancerous| —                                 | N        | C>T       |
| 11 | Male   | Cancerous    | Poorly differentiated adenocarcinoma | T        | C         |
| 12 | Male   | Paracancerous| —                                 | T        | C         |
| 13 | Male   | Cancerous    | Poorly differentiated adenocarcinoma | N        | C>T       |
| 14 | Male   | Paracancerous| —                                 | N        | C>T       |
| 15 | Female | Cancerous    | Poorly differentiated adenocarcinoma | T        | N         |

Combined with our previous RNA high-throughput sequencing, which showed the significant upexpression of autophagy-related protein, DDIT4 is a novel protein which is unknown in the field of genetic mutation.

DNA damage-inducible transcript 4 (DDIT4) was induced by a variety of stress conditions, including oxidative stress [8], endoplasmic reticulum stress [2], and hypoxia [6]. DDIT4 inhibited mammalian target of rapamycin complex 1 by stabilizing the tuberous sclerosis complex 2 [9]. DDIT4 was involved in the survival-related autophagy of cancer cell and affected targeted therapy for lung cancer [10]. DDIT4 was also connected to both autophagy and stemness, which were involved in temozolomide drug resistance and poor prognosis of glioblastoma multiforme patients [11]. The high level of DDIT4/TXNIP prooxidant complex regulated the ROS and inhibited the activity of ATG4B to control stress-induced autophagy [12]. So under the selection of harsh tumor microenvironment, the survival ability of pancreatic cancer cell with a certain type of gene mutation is an interesting question.

We speculated that DDIT4 gene mutation may be involved in pancreatic cancer cell adapted to antitumor therapy and harsh microenvironment. Mechanistically, the gene mutation and expression level of DDIT4 may affect the formation of autophagosomes, which will contribute to the survival of PDAC.
2.4. Immunoﬂuorescence. Pancreatic tissues from patients were ﬁxed with 4% paraformaldehyde, and pathological section was made. The sections were permeabilized, and non-speciﬁc binding was blocked. Primary monoclonal antibodies speciﬁc for LC3 and DDIT4 were incubated and secondary antibodies were applied for 2 hours at room temperature, and the nucleus was stained with DAPI. The stained samples were photographed with a ﬂuorescence inverted microscope in half an hour. Our immunoﬂuorescence imaging uses the same exposure time.

2.5. Statistics. Statistical differences between groups were assessed by the nonparametric Mann–Whitney U test for two groups and Kruskal-Wallis test for more than two groups. Spearman’s rank correlation coeﬃcient estimated the degree of association between two variables. Signiﬁcance was calculated at P < 0.05 by GraphPad Prism 5 (La Jolla, CA). Data are presented as mean values with standard deviation (SD) and categorical values as frequency counts and percentages. P < 0.05 was considered signiﬁcant. All data analysis was performed using SPSS statistical software version 26.

3. Results

3.1. Two Novel Gene Mutations in DDIT4 mRNA. DDIT4 is a key molecule in the signal of autophagy, and the autophagy will prevent the cancer cell for death in the harsh microenvironment. This study used retrospective case-control study to explore the correlation analysis between gene mutation sites in the tissue of pancreatic cancer. We sequenced 15 pancreatic tissues to determine the DDIT4 gene mutation in the enrolled patients (Table 2). The best pairing sequence was Homo sapiens DNA damage-inducible transcript 4 (DDIT4), mRNA (LOCUS: NM_019058); the blue one is the mutation point (m.990 and m.1246). (d) The two mutation sites of DDIT4 mRNA were located at exon 3 and 3′-UTR.

Figure 1: Gene mutation of DDIT4. (a, b) The reverse sequencing map gene of enrolled patients with pancreatic cancer. (c) The result of gene mutation blast match Homo sapiens DNA damage-inducible transcript 4 (DDIT4), mRNA (LOCUS: NM_019058); the blue one is the mutation point (m.990 and m.1246). (d) The two mutation sites of DDIT4 mRNA were located at exon 3 and 3′-UTR.
According to the NCBI data, we found that the two mutation sites were located at DDIT4 mRNA exon 3 with a portion of 3′-UTR of gene (Figure 1(d)).

3.2. The Relationship between Expression Level and Protein Localization and the 3′-UTR Mutation Style of DDIT4. DDIT4 correlated with tumor progression and affected the prognosis of patients with ovarian carcinoma [13]. In order to explore the relationship between DDIT4 and the pancreatic pathological grades, we performed IHC in pancreatic tissue. Our results indicated that DDIT4 had a higher expression level in poorly differentiated adenocarcinoma compared to other pancreatic cancer pathological styles including the moderately differentiated adenocarcinoma and false papilloma of pancreatic cancer. Under a ×400 field of view, DDIT4 was mainly located in the cytoplasm while it deepened in the nucleus. The underlying mechanism of high DDIT4 expression in poorly differentiated adenocarcinoma may be related to mutation (Figure 2(a)). In order to further clarify the correlation between 3′-UTR mutation and DDIT4 expression level, we performed IHC in pancreatic tumor tissue including several combinations of two types of genetic mutations. And then, we set up four groups (group 1: 990.T;1246.C; group 2: 990.N;1246.N; group 3: 990.N;1246.T; and group 4: 990.T>A;1246.C>T), and our result suggested that group 4 had the highest expression level (Figure 2(b)), followed by group 2 and group 3, while group 4 had the lowest expression level. Through the analysis of the results, we believed that the two DDIT4 3′-UTR gene mutations may be involved in the protein expression level. Under a ×400 field of view, DDIT4 was mainly located in the cytoplasm while it deepened in the nucleus.

3.3. The Activation of DDIT4/LC3 Signaling Pathways in Pancreatic Cancer. Previous literatures suggested that DDIT4
was closely related to autophagy in tumor cells [11]. To further clarify the correlation between DDIT4 expression level and autophagy in pancreatic cancer tissues, we performed immunofluorescence on pancreatic cancer tissues which confirmed that the expression level of LC3 and DDIT4 was significantly high in pancreatic cancer at the same time compared to the control (the low expression level of DDIT4). And we found that the expression of LC3 and DDIT4 was synchronized (Figure 3).

3.4. The Perspective View of DDIT4 in Pancreatic Cancer. Based on our experimental results and relevant literature, we summarized the relationship between DDIT4 3′-UTR mutation and pancreatic cancer. The DDIT4 gene mutation in pancreatic cancer cells leads to a base-point mutation in DDIT4 3′-UTR, and it may affect protein localization which may be involved in its biological function. The DDIT4/TX-NIP complex located in the mitochondrion promotes the expression of ROS, which inhibited the dephosphorylation ability of ATG4B. And it promotes the increased amount of LC3 expression and the activation of autophagy. DDIT4 gene mutations may result in the antitumor resistance through abnormal protein localization in the pancreatic tumor cells (Figure 4).

4. Discussion

Our article found two novel gene mutations in the 3′-untranslated region of DDIT4 mRNA in PDAC. At the same time, we found a correlation between the expression level of DDIT4 and the type of gene mutation, and DDIT4 was collocated with LC3 obviously in pancreatic cancer. This paper showed that the two novel gene mutations in the 3′-untranslated region of DDIT4 mRNA may be a novel target in pancreatic cancer.

We found two types of stable mutation in the DDIT4 mRNA 3′-UTR, and the most prevalent class of regulatory elements in the 3′-UTR is microRNA binding sites [14]. A gene mutation that resulted in a premature polyadenylation signal in CCND1 shortened its 3′-UTR and increased the risk of lymphoma [15]. Combined with our results, microRNA may be closely related to DDIT4 function in pancreatic cancer. Previous literatures revealed that miR-221 contributed to the hepatocarcinogenesis via the dysregulation of DDIT4 [16]. At the same time, miR-221 played an important role in human placental development by precisely regulating the DDIT4 expression [17]. There were some other miRNA involved in the expression of DDIT4, such as miR-495 [18], miR-30c [19], and miR-630 [20]. In summary, DDIT4 has
gene mutations in 3′-UTR in pancreatic cancer tissues, which may be related to the binding site of microRNA and affected the expression level and localization of DDIT4.

The expression levels of DDIT4 were regulated by a variety of factors, such as hypoxia [21], ionizing radiation [22], energy depletion [23], and endoplasmic reticulum stress [24]. Our results were consistent with previous study about the relationship between pathological grade and protein level in ovarian carcinoma [13]. It is possible that the poor differentiated malignancy was associated with higher rate of gene mutation. Our paper found the correlation between DDIT4 3′-UTR gene mutation and DDIT4. Mutation in genes was the most important feature in tumor, and accumulation of genetic mutation will lead to abnormal behavior of cancer cells [25]. DDIT4 3′-UTR gene mutation may affect protein localization and protein degradation by affecting the binding of DDIT4 to microRNA.

The colocalization of DDIT4 and LC3 indicated that DDIT4 was highly expressed in pancreatic cancer tissues and its expression is involved in autophagy. DDIT4 signaling was connected to both autophagy which were involved in temozolomide drug resistance and the poor prognoses of glioblastoma multiforme patients [11]. The ATF4-DDIT4-mTORC1 axis will inhibit the cancer therapy by activation of the autophagy signal pathway, and the combinatorial treatment with SIRT1/2 inhibitors and pharmacological autophagy inhibitors was an effective therapeutic strategy in lung cancer [10]. Gene mutation in DDIT4 3′-UTR will affect the autophagy by regulating the expression level of DDIT4.

The high expression of DDIT4 not only promotes the increase of autophagy in pancreatic cancer tumor cells but also may be involved in tumor resistance by inhibiting the function of immune cells around the tumor [26]. Meanwhile, the abnormal function of immune cells was involved in tumor resistance of pancreatic cancer. DDIT4 enhanced vascular inflammation and permeability in endotoxemia mice, leading to immune cell infiltration, systemic inflammation, caspase-3 activation, and apoptosis [27]. DDIT4 was related to the low level of reactive oxygen species of mitochondria in macrophages induced by IL-10 or hypoxia [28], and it inhibited the immune function of macrophages. Mast cell-activated cancer-associated fibroblasts (CAFs) and transforming growth factor-β signaling were involved in pancreatic cancer resistance to gemcitabine/nabxel [29].

**Figure 4**: Hypothetical image of DDIT4 mRNA mutation in pancreatic cancer.
therapeutic strategy targeting Wnt enhanced pancreatic cancer cytotoxicity and restored anticancer immunity in patients with nodular positive disease [30].

The heterogeneity of cancer is ultimately related to drug resistance after long-term codevelopment of tumor and microenvironment, and the mutation of drug-resistant genes is the inevitable result which benefits the survival of tumor. The UTR mutation of DDIT4 is involved in autophagy of pancreatic cancer cells by regulating the expression of DDIT4, and it may be a potential biomarker for chemotherapy resistance and poor prognosis. The overexpression of ADAM28 in pancreatic cancer is closely related to the regulation of gemcitabine resistance, so it is a new prognostic biomarker in pancreatic cancer [31]. High expression of miR-155-5p is directly associated with chemotherapy resistance and poor prognosis in PDAC patients treated with gemcitabine [32]. The mutation of autophagy-related gene DDIT4 and the higher protein expression level are of great significance. In the future, we may monitor the mutation level of DDIT4 in tissues through detecting the mutation level of DDIT4 in blood samples of pancreatic cancer patients and then realize effective assessments of chemotherapy resistance and poor prognosis.

This article has some limitations. The survival time of patients with pancreatic cancer is very short, and it is difficult to use cohort study. Therefore, this study used retrospective case-control study to explore the correlation analysis between gene mutation sites and tumor self-dependence and to provide a new target for chemotherapy resistance and prognosis in the future. This article only captured the tumor tissue at a point in time and through the comprehensive analysis of the DDIT4 gene mutations in multiple tumor samples, but the evidence is indirect. In the future, if we are lucky enough to get the same tumor patients with multiple points in time of tissue or blood samples, we can directly observe the gene mutation during the progression of the tumor.

5. Conclusion

In this study, we found a gene mutation in the 3′-UTR region of DDIT4, which may be associated with DDIT4 expression and tumor autophagy in pancreatic cancer tissues, and the further mechanistic research requires more work.

Data Availability

Data is available upon request from the authors.

Ethical Approval

All human studies were approved by the Ethics Committee of the First Affiliated Hospital of Fujian Medical University.

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors’ Contributions

Youting Chen and Feng Gao conceived and designed the experiments. Fadian Ding and Xiaoping Hong were responsible for the writing and overall progress of the article. Shirong Huang, Xianggun Fan, and Wei Lian performed the experiments. Xingting Chen, Xianggun, and Qicai Liu analyzed and interpreted the results of the experiments.

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References

[1] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2020," CA: a Cancer Journal for Clinicians, vol. 70, no. 1, pp. 7–30, 2020.
[2] M. Malvezzi, G. Carioli, P. Bertuccio et al., "European cancer mortality predictions for the year 2019 with focus on breast cancer," Annals of Oncology, vol. 30, no. 5, pp. 781–787, 2019.
[3] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2016," CA: a Cancer Journal for Clinicians, vol. 66, no. 1, pp. 7–30, 2016.
[4] J. Scott and A. Marusyk, "Somatic clonal evolution: a selection-centric perspective," Biochimica Et Biophysica Acta. Reviews on Cancer, vol. 2017, pp. 139–150, 2017.
[5] F. Wang, X. Xia, C. Yang et al., "SMAD4 gene mutation renders pancreatic cancer resistance to radiotherapy through promotion of autophagy," Clinical Cancer Research, vol. 24, no. 13, pp. 3176–3185, 2018.
[6] L. D. Wood, M. B. Yurgelun, and M. G. Goggin, "Genetics of familial and sporadic pancreatic cancer," Gastroenterology, vol. 156, no. 7, pp. 2041–2055, 2019.
[7] S. Maertin, J. M. Elperin, E. Lotshaw et al., "Roles of autophagy and metabolism in pancreatic cancer cell adaptation to environmental challenges," American Journal of Physiology. Gastrointestinal and Liver Physiology, vol. 313, no. 5, pp. G524–G536, 2017.
[8] S. S. Cho, K. M. Kim, J. H. Yang et al., "Induction of REDD1 via AP-1 prevents oxidative stress-mediated injury in hepatocytes," Free Radical Biology & Medicine, vol. 124, pp. 221–231, 2018.
[9] M. P. DeYoung, P. Horak, A. Sofer, D. Sgroi, and L. W. Ellis, "Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shunting," Genes & Development, vol. 22, no. 2, pp. 239–251, 2008.
[10] N. Mu, Y. Lei, Y. Wang et al., "Inhibition of SIRT1/2 upregulates HSPA5 acetylation and induces pro-survival autophagy via ATF4-DDIT4-mTORC1 axis in human lung cancer cells," Apoptosis, vol. 24, no. 9-10, pp. 798–811, 2019.
[11] K. H. Ho, P. H. Chen, C. M. Chou et al., "A key role of DNA damage-inducible transcript 4 (DDIT4) connects autophagy and GLUT3-mediated stemness to desensitize temozolomide
efficacy in glioblastomas,” *Neurotherapeutics*, vol. 17, no. 3, pp. 1212–1227, 2020.

[12] T. Shoshani, M. Dennis, X. Song et al., “A REDD1/TXNIP pro-oxidant complex regulates ATG4B activity to control stress-induced autophagy and sustain exercise capacity,” *Nature Communications*, vol. 6, no. 1, 2015.

[13] B. Chang, J. Meng, H. Zhu et al., “Overexpression of the recently identified oncogene REDD1 correlates with tumor progression and is an independent unfavorable prognostic factor for ovarian carcinoma,” *Diagnostic Pathology*, vol. 13, no. 1, p. 87, 2018.

[14] A. Brümmer and J. Haussér, “MicroRNA binding sites in the coding region of mRNAs: extending the repertoire of post-transcriptional gene regulation,” *BioEssays*, vol. 36, no. 6, pp. 617–626, 2014.

[15] A. Wiestner, M. Tehrani, M. Chiorazzi et al., “Point mutations and genomic deletions in CCND1 create stable truncated cyclin D1 mRNAs that are associated with increased proliferation rate and shorter survival,” *Blood*, vol. 109, no. 11, pp. 4599–4606, 2007.

[16] P. Pineau, S. Volinia, K. McJunkin et al., “miR-221 overexpression contributes to liver tumorigenesis,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 1, pp. 264–269, 2010.

[17] B. Hu, G. Xu, J. Tang et al., “microRNA221 is involved in human placental development by targeting DDIT4,” *Cellular Physiology and Biochemistry*, vol. 52, pp. 254–262, 2019.

[18] W. W. Hwang-Verslues, P. H. Chang, P. C. Wei et al., “miR-495 is upregulated by E12/E47 in breast cancer stem cells, and promotes oncogenesis and hypoxia resistance via down-regulation of E-cadherin and REDD1,” *Oncogene*, vol. 30, no. 21, pp. 2463–2474, 2011.

[19] X. H. Li, C. T. Ha, D. Fu, and M. Xiao, “Micro-RNA30c negatively regulates REDD1 expression in human hematopoietic and osteoblast cells after gamma-irradiation,” *PLoS One*, vol. 7, no. 11, article e48700, 2012.

[20] J.-X. Cao, Y. Lu, J.-J. Qi et al., “MiR-630 inhibits proliferation by targeting CDC7 kinase, but maintains the apoptotic balance by targeting multiple modulators in human lung cancer A549 cells,” *Cell Death & Disease*, vol. 5, no. 9, article e1426, 2014.

[21] T. Shoshani, A. Faerman, I. Mett et al., “Identification of a novel hypoxia-inducible factor 1-responsive gene, RTP801, involved in apoptosis,” *Molecular and Cellular Biology*, vol. 22, no. 7, pp. 2283–2293, 2002.

[22] L. W. Ellisen, K. D. Ramsayer, C. M. Johanssens et al., “REDD1, a developmentally regulated transcriptional target of p63 and p53, links p63 to regulation of reactive oxygen species,” *Molecular Cell*, vol. 10, no. 5, pp. 995–1005, 2002.

[23] A. Sofer, K. Lei, C. M. Johanssens, and L. W. Ellisen, “Regulation of mTOR and cell growth in response to energy stress by REDD1,” *Molecular and Cellular Biology*, vol. 25, no. 14, pp. 5834–5845, 2005.

[24] S. R. Kimball and L. S. Jefferson, “Induction of REDD1 gene expression in the liver in response to endoplasmic reticulum stress is mediated through a PERK, eIF2α phosphorylation, ATF4-dependent cascade,” *Biochemical and Biophysical Research Communications*, vol. 427, no. 3, pp. 485–489, 2012.

[25] J. H. Bushweller, “Targeting transcription factors in cancer – from undruggable to reality,” *Nature Reviews. Cancer*, vol. 19, no. 11, pp. 611–624, 2019.