Case Report: Novel RPGRIP1L Gene Mutations Identified by Whole Exome Sequencing in a Patient With Multiple Primary Tumors

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A 78 years old Chinese woman with five different cancer types and a family history of malignancy was the subject of this study. Pancreatic adenocarcinoma and gingival squamous cell carcinoma tissues were obtained from the patient and sequenced using Whole Exome Sequencing. Whole exome sequencing identified 20 mutation sites in six candidate genes. Sanger Sequencing was used for further validation. The results verified six mutations in three genes, OBSCN, TTN, and RPGRIP1L, in at least one cancer type. Immunohistochemistry was used to verify protein expression. mRNA expression analysis using The Cancer Genome Atlas database revealed that RPGRIP1L was highly expressed in several cancer types, especially in pancreatic adenocarcinoma, and correlated with patient survival and sensitivity to paclitaxel, probably through the TGF-β signaling pathway. The newly identified somatic mutations in RPGRIP1L might contribute to pathogenesis in the patients. Protein conformation simulation demonstrated that the alterations had caused the binding pocket at position 708 to change from concave to convex, which could restrict contraction and extension, and interfere with the physiological function of the protein. Further studies are required to determine the implication of RPGRIP1L in this family and in multiple primary tumors.

Keywords: RPGRIP1L, multiple primary tumors, somatic mutation, pancreatic adenocarcinoma, whole-exome sequencing

INTRODUCTION

Multiple primary tumors (MPTs) are a phenomenon rarely clinically seen. MPTs are defined as two or more malignancies simultaneously or successively occurring in an individual (Vogt et al., 2017). The incidence of MPTs is higher in the elderly, especially in people aged 50–60 (Patrascu et al., 2010). Despite understanding that multiple factors including unhealthy lifestyle (Hori et al., 2011), chemoradiotherapy (Oeffinger et al., 2013), and genetic mutations (Park et al., 2014), are involved in the development of MPTs, the pathogenesis of MPTs remains unclear. Deleterious germline mutations and defects in DNA repair genes are closely related to MPTs (Tiwari et al., 2016). Recently, Xu et al. identified a homozygous germline insertion mutation in WWOX, a DNA repair-related gene, from a 26 years old female patient with MPTs (Xu et al., 2019). Nevertheless, further investigation is required to clarify the molecular mechanisms underlying MPTs.
MATERIALS AND METHODS
Clinical Information and Samples in the Study
The patient is a 78 years old Chinese woman, who had successively developed five different cancer types. This patient developed hepatocellular carcinoma in 2000 (for which she received interventional therapy in the liver), colorectal adenocarcinoma in 2002 (for which she received a partial colon resection), invasive breast carcinoma in 2011 (for which she received radical mastectomy of the left breast), gingival squamous cell carcinoma in 2018 (for which she received right side gingival tumor resection), and pancreatic adenocarcinoma in 2018 (for which she received a partial pancreatectomy). Based on the patients' report, none of the cancers were treated with radiotherapy or chemotherapy. The patient was admitted with a right cervical mass in November 2019 and mass biopsy was performed in The Affiliated Huaian

| Gene name | Location | Primer | Primer sequences (5′-3′) | Size (bp) |
|-----------|----------|--------|--------------------------|----------|
| MUC16     | chr19:9050161 | C1-F   | GTTGTGATCATCATTTCTGGG    | 261      |
|           |          | C1-R   | TAACTATGTCAGCTCAATTTCTGGG|          |
| MUC16     | chr19:9050163 | C1-F   | GTTGTGATCATCATTTCTGGG    | 261      |
|           |          | C1-R   | TAACTATGTCAGCTCAATTTCTGGG|          |
| MUC16     | chr19:9058599 | C2-F   | TGGGTGGGTGATGTGTTATTTCTG| 291      |
|           |          | C2-R   | TGGGTGGGTGATGTGTTATTTCTG|          |
| TTN       | chr2:179434170 | C9-F   | GTGGTTTCTGTTGACTTTCAAGTT | 283      |
|           |          | C9-R   | TAAATATAATGGGCAACAGGCAAAAGTG|      |
| TTN       | chr2:179500799 | C10-F  | GAAATCTCCCAGGAAATAGTACTAACC| 274      |
|           |          | C10-R  | GAAGAGGAGTACGAGTACGAGTAC|          |
| TTN       | chr2:179640476 | C11-F  | GACCTAGTTCATTCATCTGATGTT | 289      |
|           |          | C11-R  | TGAAGTTCAGAAAGGCTGAAAAGAG|          |
| RGSL1     | chr1:182443294 | C12-F  | TATACAAATGGCTCCTGCCAGAT| 347      |
|           |          | C12-R  | CCTGCCAGAGGTCAAGAGGTT   |          |
| RGSL1     | chr1:182443296 | C12-F  | TATAACAAATGGCTCCTGCCAGAT| 347      |
|           |          | C12-R  | CCTGCCAGAGGTCAAGAGGTT   |          |
| RGSL1     | chr1:182499947 | C13-F  | CCATTACCACTTGAGAGGTCTCT | 250      |
|           |          | C13-R  | ACTGTTTCCTCATCTAACTGGTG|          |
| RGSL1     | chr1:182499952 | C13-F  | CCATTACCACTTGAGAGGTCTCT | 250      |
|           |          | C13-R  | ACTGTTTCCTCATCTAACTGGTG|          |
| OBSCN     | chr1:228399569 | C14-F  | AAAATATGAGGTGGAGAGGTGTTG | 341      |
|           |          | C14-R  | GCCAAGCTTCAAGATGAAGAGGAG|          |
| OBSCN     | chr1:228404900 | C15-F  | TTTGAGTGAGTGAACCTGCGAAG | 195      |
|           |          | C15-R  | GAGCCGGAGATCACAGAGGAG    |          |
No.1 People's Hospital of Nanjing Medical University. The pathology results showed metastatic squamous cell carcinoma that had originated from the gingival tumor and the patient received chemoradiotherapy in our hospital. Moreover, the patient had a very complicated family history of malignancy, which showed that the patient's mother died of esophageal squamous cell carcinoma, and her two brothers (the patient's uncles) also died of malignancies (with no exact details). The patient had two older sisters, two older brothers, and a younger brother. Her oldest brother died of leukemia, and her second oldest brother, who had a history of cardia carcinoma, was alive. Her younger brother died of cardia carcinoma and had a history of gingival squamous cell carcinoma. However, the rest of her family, including her father, two sisters, and her four children, had no cancer history (Supplementary Figure 1).

Therefore, it is valuable to discuss the etiology and pathogenesis of this case. This study was approved by the Clinical Research Ethics Committee of The First Affiliated Hospital of University of Science and Technology of China (Protocol number: P-015). The patient and her family members provided written informed consent for study participation. A written, informed consent was obtained from the participant for the publication of this case report. Our study followed the institutional ethical guidelines approved by The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University (Huai’an, China).

**DNA Extraction**

Sample loss and damage meant that only two cancer samples (gingival and pancreatic) were available for further sequencing. The patient's blocks of gingival squamous cell carcinoma and pancreatic adenocarcinoma after resection, as well as her peripheral blood samples were collected. Genomic DNA of the samples was isolated using the FastPure FFPE DNA Isolation Kit (Vazyme, Nanjing, China), and purified using 1% Sepharose electrophoresis. DNA purity and concentration were measured using a NanoPhotometer® spectrophotometer (IMPLEN, CA, USA) and Qubit® 3.0 Flurometer (Life Technologies, CA, USA).

**Whole Exome Sequencing and Data Analysis**

To prepare the Illumina sequencing libraries, the SureSelect Human All Exon kit V6 (Agilent Technologies, Santa Clara, CA, USA) was used. Genomic DNA samples (3 µg) were randomly fragmented into 150–200 bp fragments using a Covaris S2
DNA fragments had deoxyadenosine bases added to their 3′ system (Agilent Technologies, Santa Clara, CA, USA). In brief, preparation according to the SureSelect XT Target Enrichment sonicator (Covaris, Woburn, MA, USA) and subjected to library sequencing. Among the 20 mutation sites, five of them were close to another five target cancers were amplified using PCR Master Mix (Illumina, San Diego, CA, USA) and subjected to Sanger sequencing. Among the 20 mutation sites, five of them were close to another five target sites which could be amplified by the same primers. Therefore, we designed 15 primer pairs. Sequencing primers were designed using Primer3 (v.0.4.0) software and were generated by Sangon Biotech (Shanghai, China; Table 1).

### Immunohistochemistry

Samples of the patients’ pancreatic adenocarcinoma and gingival squamous cell carcinoma were further analyzed by immunohistochemistry. Paraffin-embedded sections were deparaffinized and rehydrated, and sections were covered with Tris-EDTA (TE) buffer and heated for 10 min for antigen retrieval. Sections were incubated overnight at 4°C with an anti-RPGRIP1L antibody (1:200; 55160-1-AP, Proteintech, Wuhan, China), rinsed with phosphate buffered saline, and incubated with a secondary antibody (anti-rabbit) at 37°C for 30 min. The sections were finally incubated with 3,3′-diaminobenzidine and stained with hematoxylin. RPGRIP1L expression was identified by immunohistochemistry. Paraffin sections were analyzed with a software program (Bioinformatics Analysis).

### Bioinformatics Analysis

The Cancer Genome Atlas (TCGA) database was used to verify the expression of candidate genes in different cancers. The prognostic value of the hub genes was analyzed by TCGA, KM-plotter and an online tool called OncoLnc (http://www.oncolnc.org/). The pancreatic adenocarcinoma patients from the TCGA database were divided into two groups at the optimal cut-off point. The expression of candidate genes in different cancers. The TCGA, KM-plotter and an online tool called OncoLnc (http://www.oncolnc.org/). The pancreatic adenocarcinoma patients from the TCGA database were divided into two groups at the optimal cut-off point. The expression of candidate genes in different cancers. The TCGA, KM-plotter and an online tool called OncoLnc (http://www.oncolnc.org/).

### Sanger Sequencing Validation

Shared non-synonymous SNVs and InDels identified in the two cancers were amplified using PCR Master Mix (Illumina, San Diego, CA, USA) and subjected to Sanger sequencing. Among the 20 mutation sites, five of them were close to another five target sites which could be amplified by the same primers. Therefore, we designed 15 primer pairs. Sequencing primers were designed using Primer3 (v.0.4.0) software and were generated by Sangon Biotech (Shanghai, China; Table 1).

### Table 3: Summary of mutations verified by Sanger sequencing.

| Primer | Gene name | Location | Ref | Alt | YKD1489-A genotype | YKD1489-B genotype |
|--------|-----------|----------|-----|-----|-------------------|-------------------|
| 1      | MUC16     | chr:19:9050161 | -   | AA  | ++/+              | ++/+              |
| 1      | MUC16     | chr:19:9050163 | -   | CCACCTCAAGAGCC | ++/+              | ++/+              |
| 2      | MUC16     | chr:19:9058599 | -   | TGCC | ++/+              | ++/+              |
| 2      | MUC16     | chr:19:9058600 | -   | CTCCTGAGGCTCCTCAG | ++/+              | ++/+              |
| 3      | MUC16     | chr:19:9065157 | -   | CTCACACCA | ++/+              | ++/+              |
| 4      | MUC16     | chr:19:9078232 | -   | ACTCCTGACTGAGACACTGAGATCTCATGTCAT | ++/+              | ++/+              |
| 5      | RPGRIP1L  | chr:16:53886476 | C   | A   | ++/+              | mut/+             |
| 5      | RPGRIP1L  | chr:16:53886477 | C   | A   | ++/+              | mut/+             |
| 6      | RPGRIP1L  | chr:16:53886660 | -   | AGACAACCTCAAGTCAGTG | ++/+              | ++/+              |
| 7      | ERICH3    | chr:1.75037773 | -   | CCTGAAGGAAAGGACCGGCAAGATGAGAG | ++/+              | ++/+              |
| 7      | ERICH3    | chr:1.75055676 | -   | GACAGGGGAAAGGCAACACTGACA | ++/+              | ++/+              |
| 8      | TTN       | chr:2:179434170 | -   | A   | ++/+              | mut/+             |
| 10     | TTN       | chr:2:179500799 | C   | G   | ++/+              | ++/+              |
| 11     | TTN       | chr:2:179640476 | G   | C   | ++/+              | ++/+              |
| 12     | RGS1      | chr:1:82443294 | -   | TCTTGAGGAGCTG | ++/+              | ++/+              |
| 12     | RGS1      | chr:1:82443296 | -   | TGACAAATGAGGATGA | ++/+              | ++/+              |
| 13     | RGS1      | chr:1:82499947 | -   | AA  | ++/+              | ++/+              |
| 13     | RGS1      | chr:1:82499952 | -   | GCCACTCGAGGCTAGAGGGGAGGATGACGCGTGACG | ++/+              | ++/+              |
| 14     | OBSCN     | chr:1:228399669 | C   | T   | ++/+              | mut/+             |
| 15     | OBSCN     | chr:1:228404900 | -   | GGTGAAGGCTGCTGAGGCAACAOCC | ++/+              | ++/+              |

+/+, wild type; mut/+, mutant type. YKD1489-A, gingival squamous cell carcinoma; YKD1489-B, pancreatic adenocarcinoma.
Protein Conformation Simulation
The RPGRIP1L structure information was obtained from the UniProt database (https://ebi10.uniprot.org/uniprot/Q68CZ1/), which showed existing crystal structure data obtained using the NMR method (PDB Entry: 2YRB, Chain: A, Positions: 595–737). The NMR structure of RPGRIP1L was acquired from the PDB database. The PRGRIP1L amino acid alterations were p.G708C and p.G708V. Protein conformation simulations were performed using the SWISS-MODEL online tool (https://swissmodel.expasy.org/). PyMOL software was used for structure visualization.

RESULTS
Five Distinct Cancers Successively Developed in an Individual Patient
This 78-year-old Chinese woman was first admitted to our hospital on November 2nd, 2019 with a right cervical mass. This mass was confirmed to be the result of metastasis of her previously diagnosed gingival squamous cell carcinoma. While taking her history, we discovered that she had a history of five different cancer types and a complicated family history. The five different cancer types were hepatocellular carcinoma in 2000, colorectal adenocarcinoma in 2002, invasive breast carcinoma in 2011, gingival squamous cell carcinoma in 2018, and pancreatic adenocarcinoma in 2018. Unfortunately, all of her cancers were treated in different hospitals and the first three cancer sample blocks were lost or damaged. Diagnosis of her first three cancers was based on her previous medical records and imaging examinations. The latter two cancer types, gingival squamous cell carcinoma and pancreatic adenocarcinoma, were confirmed by immunohistochemical methods (Figure 1).

Candidate Variants Detected From WES
DNA extracted from the patient's samples was sequenced using WES. The results of her peripheral blood were applied to deduction of background variants. This identified 221 SNVs and InDels in her gingival squamous cell carcinoma and 281 SNVs and InDels in her pancreatic adenocarcinoma. Her peripheral blood results were used as a control. Comparison and analysis of the results revealed six common candidate genes with 20 mutation sites for further validation (Table 2).
TABLE 4 | The sources of IHC pictures obtained from HPA.

| Antibody HA065801 | Liver not detected: https://images.proteinatlas.org/65801/167889_A_7_4.jpg | LiHC low: https://images.proteinatlas.org/65801/167889_B_9_3.jpg |
|-------------------|------------------------------------------------|----------------|
|                   | COLON not detected: https://images.proteinatlas.org/65801/167889_A_2_2.jpg | COAD low: https://images.proteinatlas.org/65801/167886_A_2_2.jpg |
|                   | BREAST not detected: https://images.proteinatlas.org/65801/167889_B_2_4.jpg |                     |
|                   | BRC A high: https://images.proteinatlas.org/65801/167889_A_8_1.jpg | ORAL MUCOSA not detected: https://images.proteinatlas.org/65801/167889_A_8_1.jpg |
|                   | HNSC low: https://images.proteinatlas.org/65801/167877_A_2_8.jpg |                     |
|                   | PANCREAS low: https://images.proteinatlas.org/65801/167889_A_1_3.jpg | PANCREAS not detected: https://images.proteinatlas.org/65801/165308_A_3_3.jpg |
|                   | PAAD medium: https://images.proteinatlas.org/65801/167888_B_6_7.jpg |                     |
| TTN (Antibody HA07042) | Liver not detected: https://images.proteinatlas.org/7042/165308_A_7_4.jpg | LiHC medium: https://images.proteinatlas.org/7042/165306_B_9_7.jpg |
|                   | COLON not detected: https://images.proteinatlas.org/7042/165308_A_8_3.jpg | COAD medium: https://images.proteinatlas.org/7042/165304_A_2_5.jpg |
|                   | BREAST not detected: https://images.proteinatlas.org/7042/165308_B_2_4.jpg | BRC A not detected: https://images.proteinatlas.org/7042/165304_A_6_8.jpg |
|                   | ORAL MUCOSA low: https://images.proteinatlas.org/7042/165308_A_9_1.jpg | ORAL MUCOSA not detected: https://images.proteinatlas.org/7042/165308_A_3_3.jpg |
|                   | HNSC medium: https://images.proteinatlas.org/7042/165328_A_2_6.jpg | PAAD medium: https://images.proteinatlas.org/7042/165306_B_5_2.jpg |
|                   | PANCREAS not detected: https://images.proteinatlas.org/7042/165308_A_3_3.jpg |                     |

RPGRIP1L (Antibody HA039405) | PANCREAS medium: https://images.proteinatlas.org/39405/96534_A_2_3.jpg | LiHC medium: https://images.proteinatlas.org/39405/96535_B_6_7.jpg |
|                   | PAAD high: https://images.proteinatlas.org/39405/96534_B_6_7.jpg | COLON not detected: https://images.proteinatlas.org/39405/96534_A_2_3.jpg |
|                   | LIVER medium: https://images.proteinatlas.org/39405/96534_A_9_4.jpg | LiHC high: https://images.proteinatlas.org/39405/96535_B_9_8.jpg |
|                   | LiHC high: https://images.proteinatlas.org/39405/96535_B_6_7.jpg | COAD medium: https://images.proteinatlas.org/39405/96534_A_2_3.jpg |
|                   | LIVER medium: https://images.proteinatlas.org/39405/96534_A_9_4.jpg | BREAST not detected: https://images.proteinatlas.org/39405/96534_B_2_4.jpg |
|                   | LiHC high: https://images.proteinatlas.org/39405/96535_B_9_8.jpg | BRC A high: https://images.proteinatlas.org/39405/96534_A_6_3.jpg |
|                   | ORAL MUCOSA low: https://images.proteinatlas.org/39405/96534_A_9_1.jpg | ORAL MUCOSA not detected: https://images.proteinatlas.org/39405/96534_A_9_1.jpg |
|                   | HNSC medium: https://images.proteinatlas.org/39405/96534_B_9_1.jpg | HNSC low: https://images.proteinatlas.org/39405/96534_A_7_1.jpg |

IHC, immunohistochemistry; HPA, Human Protein Atlas; LiHC, liver hepatocellular carcinoma; COAD, colon adenocarcinoma; BRCA, breast invasive carcinoma; HNSC, head and neck squamous cell carcinoma; PAAD, pancreatic adenocarcinoma. The HNSC paired normal tissues are from human oral mucosa.

Bioinformatic Analyses Identify RPGRIP1L as a Potential Susceptibility Gene

Bioinformatic analyses were applied to explore the role of RPGRIP1L in pancreatic adenocarcinoma. In combination with the TCGA dataset, expression analysis revealed that RPGRIP1L mRNA was significantly upregulated in 179 pancreatic adenocarcinoma patients, Figure 4A. The patients were then divided into two groups based on the minimum P-value of the curve. Using the Kaplan-Meier Plotter (KM, http://www.kmplot.com/) tool, survival analysis showed that RPGRIP1L expression was closely related to survival in patients with pancreatic adenocarcinoma (Figure 4B). Next, we performed GESA (Subramanian et al., 2005) and GSVA (Hanzelmann et al., 2013). The GESA results showed that RPGRIP1L expression correlated with three pathways: circadian rhythm, ECM-receptor interaction, and TGF-β signaling pathways (Figures 4D,F). GSVA showed that RPGRIP1L expression was associated with several biological processes including apoptosis, angiogenesis, and in part of classic signaling pathways involved in cancer development including the TGF-β, KRAS, PI3K-Akt-mTOR, p53, and Wnt/β-catenin signaling pathways (Figure 4E). As chemotherapy is commonly used to treat pancreatic adenocarcinoma, we estimated the chemotherapeutic response of patients with pancreatic adenocarcinoma using the R package “pRrophetic” (Geeleher et al., 2014). This analysis was based on the largest pharmacogenomics database GDSC (https://www.cancerrxgene.org/). This estimation was based on the half maximal inhibitory concentration (IC50), which revealed that RPGRIP1L expression was markedly associated with paclitaxel, one of the most widely used clinical anti-tumor drugs in cancer chemotherapy (Figure 4C). Protein conformation simulation was conducted using information from UniProt and PDB databases, and revealed that, in this patient, the glycine (Gly) at RPGRIP1L protein position 708 had changed to cysteine (Cys) or valine (Val) due to the somatic mutations in RPGRIP1L. The simulation demonstrated that these alterations changed

Validation of Mutation Sites by Sanger Sequencing

WES analysis identified a total of 20 variants in six genes, ERICH3, OBSCN, RGSL1, TTN, RPGRIP1L, and MUC16. These variants were subjected to confirmation using Sanger sequencing. Sanger Sequencing results confirmed two mutation sites in RPGRIP1L, one of TTN, and one of OBSCN in the patient's pancreatic adenocarcinoma, and two mutation sites in TTN in her gingival squamous cell carcinoma (Table 3).

Expression of the RPGRIP1L Protein in Cancers

Sanger sequencing confirmed that RPGRIP1L, TTN, and OBSCN were mutated in this patient. We used the Human Protein Atlas (https://www.proteinatlas.org/) to analyze the level of protein expression from the three genes in multiple cancers. This analysis showed that RPGRIP1L is highly expressed in liver cancer, breast cancer, and pancreatic cancer, while expression of TTN and OBSCN was not markedly observed (Figure 2). The image sources are listed in Table 4. We also analyzed RPGRIP1L, TTN, and OBSCN mRNA expression using Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia2.cancer-pku.cn/) tools. Results of this analysis were consistent with the observed protein expression results and showed that RPGRIP1L mRNA was more highly expressed than both TTN, and OBSCN mRNA in cancers (Figure 4A). Identification of high levels of RPGRIP1L expression in pancreatic cancer prompted us to extend this finding by analyzing RPGRIP1L protein expression in the patients' cancer samples. Immunostaining for RPGRIP1L in her pancreatic cancer tissues revealed predominantly cytoplasmic staining in most of the cancer cells, which demonstrated that RPGRIP1L protein expression was higher in her pancreatic adenocarcinoma tissues than adjacent pancreatic tissues, but was slightly lower in her gingival squamous cell carcinoma tissues than in normal gingival tissues (Figure 3).

Frontiers in Genetics | www.frontiersin.org 6 February 2021 | Volume 12 | Article 620472

Guo et al. RPGRIP1L Mutations by WES
the binding pocket structure from concave to convex. This could restrict contraction and extension, and interfere with the physiological function of the protein (Figure 5).

**DISCUSSION**

This study investigated the molecular alterations in a case of MPTs. RPGRIP1L was identified as a candidate gene with nonsynonymous SNVs (chr16:53686476, C>A; chr16:53686477, C>A) as a somatic mutation candidate. Moreover, bioinformatic analyses revealed that RPGRIP1L expression was related to patient survival and chemosensitivity to paclitaxel in pancreatic adenocarcinoma, presumably occurring through the TGF-β signaling pathway.

Recently, great achievements have been made in the diagnosis and treatment of cancers. These advances have contributed to more satisfactory survival times and higher incidence of MPTs. It is generally accepted that inherited genetic defects are most likely to cause MPTs (Chan et al., 2018). A recent paper reported a rare variant within a PARP4 pseudogene (PARP4P2) in a patient with MPTs and familial cancer history. They suggested that the PARP4P2 pseudogene variant could induce PARP4 down-regulation, which might confer susceptibility to the development of multiple metachronous cancers (Cirello et al., 2019). In this study, our results represent newly discovered somatic mutations in the RPGRIP1L gene of a patient with MPTs and family history.

The retinitis pigmentosa GTPase regulator interacting protein 1-like (RPGRIP1L), also known as Ftm, localizes to the basal body-centrosome complex or to primary cilia and centrosomes in ciliated cells, is highly conserved (Wiegering et al., 2018). RPGRIP1L negative embryos show a variety of defects caused by cilia dysfunction (Vierkotten et al., 2007; Gerhardt et al., 2015). Defects in this gene can affect the development of several organs (Chen et al., 2015; Andreu-Cervera et al., 2019; Wang et al., 2019) and result in multiple diseases, including Joubert syndrome (JBTS) (Arts et al., 2007) and Meckel syndrome (MKS) (Delous et al., 2007). Previous studies revealed that RPGRIP1L plays an important role in the assembly of the transition zone, a region of
**FIGURE 4 |** Bioinformatic analyses of RPGRIP1L gene expression and function. (A) Analysis of OBSCN, TTN, and RPGRIP1L mRNA expressions in five cancer types from TCGA database. The red and green colors of cancer types mean significantly difference (red, upregulated in tumor; green, downregulated in tumor, all compared with normal tissues). (B) The RPGRIP1L expression is related with PAAD patients’ survival. (C) The RPGRIP1L expression is related with patients’ sensitivity to paclitaxel. (D) GESA results showed that the RPGRIP1L expression was correlated with three pathways including circadian rhythm, ECM-receptor interaction, and TGF-β signaling pathways. (E) GSVA showed that RPGRIP1L expression was associated with several biological processes. Blue part means low expression, green part means high expression, gray part means no difference of RPGRIP1L expression. (F) GESA results of predicted RPGRIP1L binding targets in circadian rhythm, ECM-receptor interaction, and TGF-β signaling pathways. TCGA, The Cancer Genome Atlas; PAAD, pancreatic adenocarcinoma; GESA, gene-set enrichment analysis; GSVA, gene set variation analysis.
the cilia (Jensen et al., 2015). **RPGRIP1L** was previously shown to govern the function of the Proteasome 26S Subunit, Non-ATPase 2 (PSMD2) by interacting with it and controlling the ciliary signaling through affecting ciliary proteasome activity (Gerhardt et al., 2015). Furthermore, it was recently demonstrated that **RPGRIP1L** deficiency impairs Hedgehog (Hh)/Gli signaling. **RPGRIP1L** can region-specifically influence mouse forebrain development through Hh signaling (Andreu-Cervera et al., 2019). **RPGRIP1L** was also reported to affect autophagy activity. Struchtrup et al. reported that absence of **RPGRIP1L** inhibits the initiation and later steps in the autophagy process, e.g., the generation of autophagosomes, which occurs via cilia-mediated mTOR signaling activation (Struchtrup et al., 2018). Recently, mutations in **RPGRIP1L** were reported in obesity and brain function. One study showed that rs13334070, in **RPGRIP1L** intron 4, has a significant association with obesity (Javanrouh et al., 2019). Reble et al. recently identified a SNV, rs7203525, that influences an alternative splicing event in **RPGRIP1L**, increasing exon 20 inclusion and potentially impacting brain function (Reble et al., 2020).

Using WES, our study discovered two novel **RPGRIP1L** SNVs in a case with MPTs, which were further validated by Sanger sequencing. Since the two SNVs were found only in the pancreatic adenocarcinoma tissues, and that the mutations occurred in exons, they are likely to influence translation efficiency, alternative splicing, and DNA copy number (El Marabti and Younis, 2018), and may be related to **RPGRIP1L** protein expression levels. However, the relationship between the SNVs and the observed expression differences needed to be validated further.

Previous studies had reported downregulation of **RPGRIP1L** in human hepatocellular carcinoma and suggested it was a tumor suppressor gene. Downregulation of **RPGRIP1L** increased Mitotic arrest deficient 2 (Mad2) protein levels, resulting in tumor cell transformation (Lin et al., 2009). Moreover, interaction between **RPGRIP1L** and Myosin Va, which was reported to be increased in several cancers, was detected at the ciliary base, indicating that **RPGRIP1L** might regulate the amount of Myosin Va and suppress tumorigenesis (Assis et al., 2017; Wiegering et al., 2018). Conversely, based on the above analysis and description, we contended that **RPGRIP1L** might act as a tumor promoter gene in pancreatic adenocarcinoma, since high **RPGRIP1L** expression was observed, and was closely related to patient’s survival, TGF-β signaling pathway, and sensitivity to the chemotherapeutic drug paclitaxel. Additionally, protein conformation simulation analysis revealed that the identified mutations might affect the binding ability of the protein and impact downstream targets. In combination with the previous studies, our results suggest that **RPGRIP1L** might play a promotor or suppresser role in a cancer type-specific manner.

Finally, our study has some limitations. Samples of the patient’s other three cancer tissues were not available, and the novel **RPGRIP1L** mutations were not found in gingival squamous cell carcinoma. One conceivable explanation is that her gingival squamous cell carcinoma might be caused by dietary habit and lack of oral care. Indeed, people from this region have a higher incidence of esophageal squamous cell carcinoma because of their preference for pickled and hot foods. The patient also suffered from diabetes, which appeared to be linked to oral cancer (Mekala et al., 2020). Moreover, samples from her family members were not available as well. Due to her complicated family history of malignancy, we could not exclude the possibility that there were germline mutations inherited by the patient. Further investigations are required to clarify the potential role of **RPGRIP1L** mutations in tumorigenesis and its value as a therapeutic target.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by Clinical Research Ethics Committee of The First Affiliated Hospital of University of Science and Technology of China. The patients/participants provided their written informed consent to participate in this study.
AUTHOR CONTRIBUTIONS

MH and XS designed the research. JG and YY performed the experiments. ZJ provided the clinical samples. ZJ, MY, and XX analyzed and interpreted the data. JG wrote the manuscript. MH critically commented and edited the manuscript. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

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Wang, L., De Solis, A. J., Goffer, Y., Birkenbach, K. E., Engle, S. E., Tanis, R., et al. (2019). Ciliary gene RPGRIP1L is required for hypothalamic arcuate neuron development. *JCI Insight* 4:123337. doi: 10.1172/jci.insight.123337

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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