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(56) Documents Cited:
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CN 116004810 A CN 115678995 A
CN 101541977 A US 20140342946 A1
Guo,X.L et al TMED3 Promotes the Development of Malignant Melanoma by Targeting CDCA8 and Regulating P13K/Akt Pathway Cell & Bioscience, Vol.vol.13.29-03-2023, ARTICLE 65,abstract

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Other: CJFD, CNTXT, ENTXT, ENTXTC, STN, PUBMED, CNKI, NCBI, WANFANG:SEQ ID NOs:1-5,TMED8

(54) Title of the Invention: **Use of TMED8 gene in preparation of drug for treating tumor and kit for diagnosing tumor**
Abstract Title: **Use of TMED8 gene in preparation of drug for treating tumor and kit for diagnosing tumor**

(57) The present application belongs to the technical field of precision medicine of tumors. Disclosed is the use of a TMED8 gene in the preparation of a drug for treating a tumor and a kit for diagnosing the tumor. The up-regulation of TMED8 gene expression can significantly inhibit the migration and proliferation abilities of pancreatic cancer cells, and can promote the apoptosis of pancreatic cancer cells; also, the TMED8 gene is related to an MAPK signaling pathway. Therefore, TMED8 can be used as an effective target for the gene diagnosis and treatment of pancreatic cancer. The expression of TMED8 in pancreatic cancer, the relationship between TMED8 and the prognosis and survival of patients, and the effect of TMED8 on the biological behavior of pancreatic cancer cells are studied, and it is explored that the action mechanism of TMED8 may be closely related to MAPK signaling pathway. TMED8 can be used as a kit for diagnosing pancreatic cancer and a drug for treating pancreatic cancer, which provides effective support for the early diagnosis and treatment of pancreatic cancer.

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This international application has entered the national phase early. As it had not been published nor had an international search been conducted at early entry, it is published under section 16 and has been searched under section 17

DRAWINGS

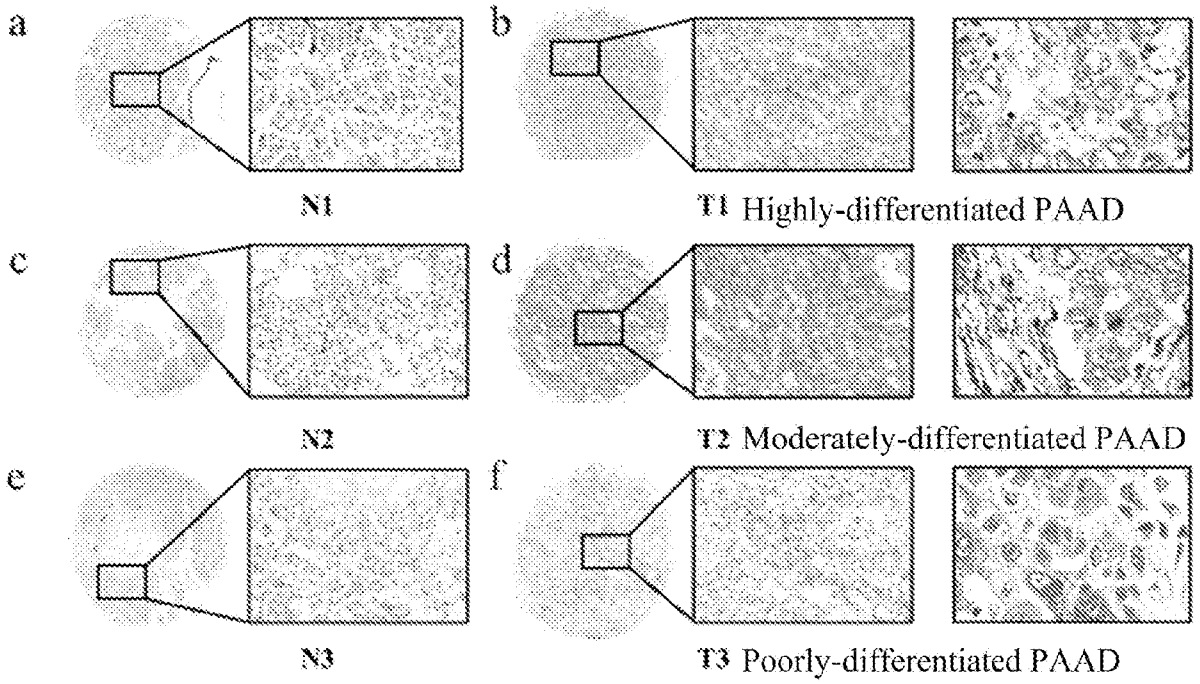


FIG. 1

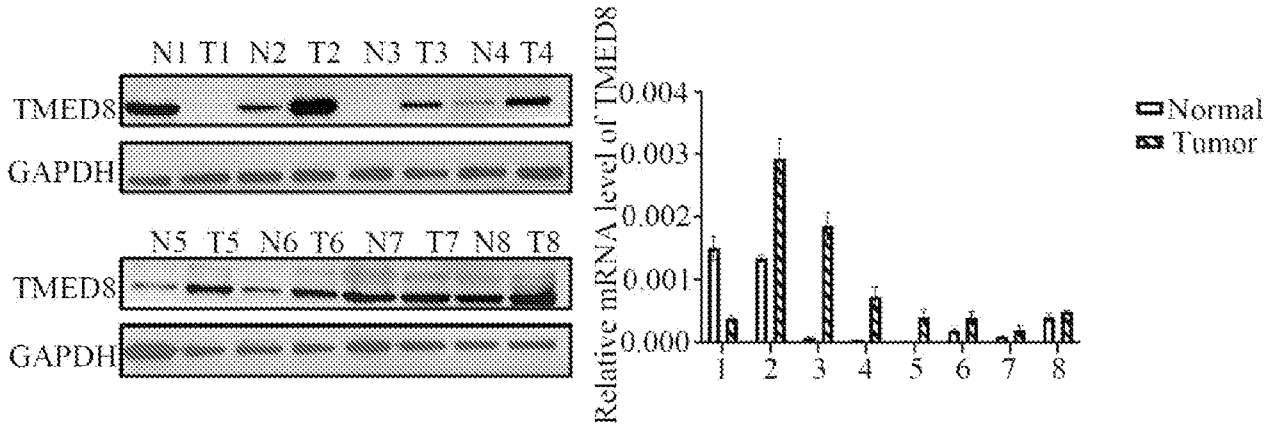


FIG. 2

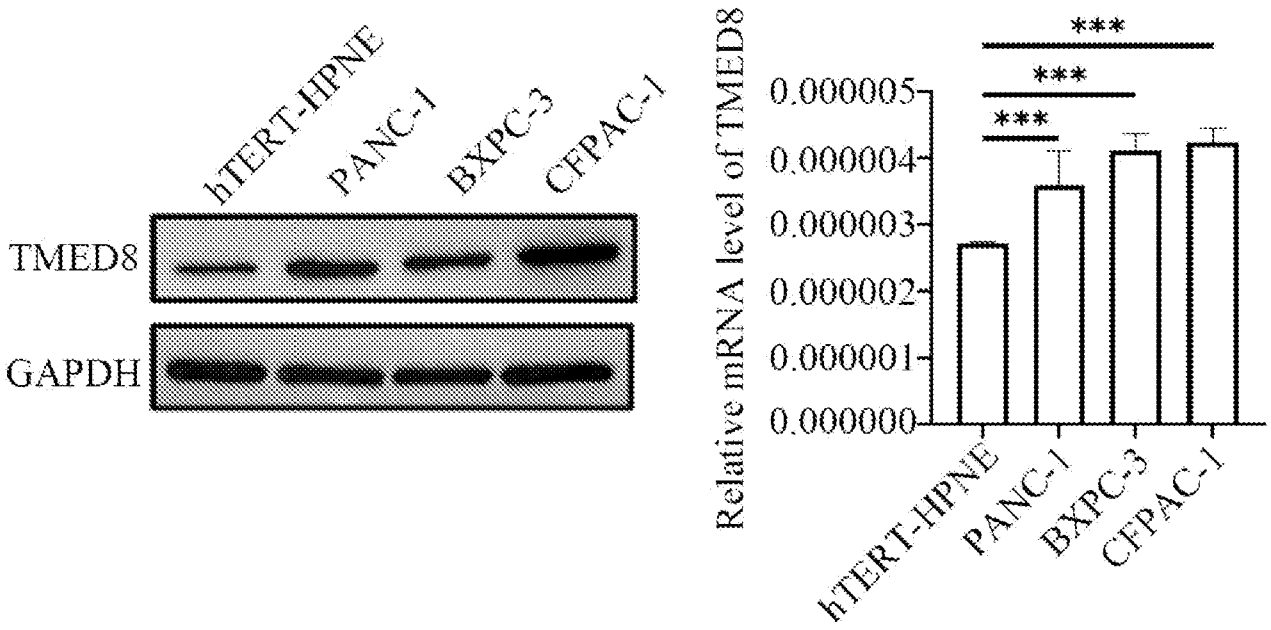

hTERT-HPNE
PANC-1
BXPC-3
CFPAC-1
TMED8
GAPDH
Relative mRNA level of TMED8
0.000005
0.000004
0.000003
0.000002
0.000001
0.000000
***
***
***
hTERT-HPNE
PANC-1
BXPC-3
CFPAC-1

FIG. 3

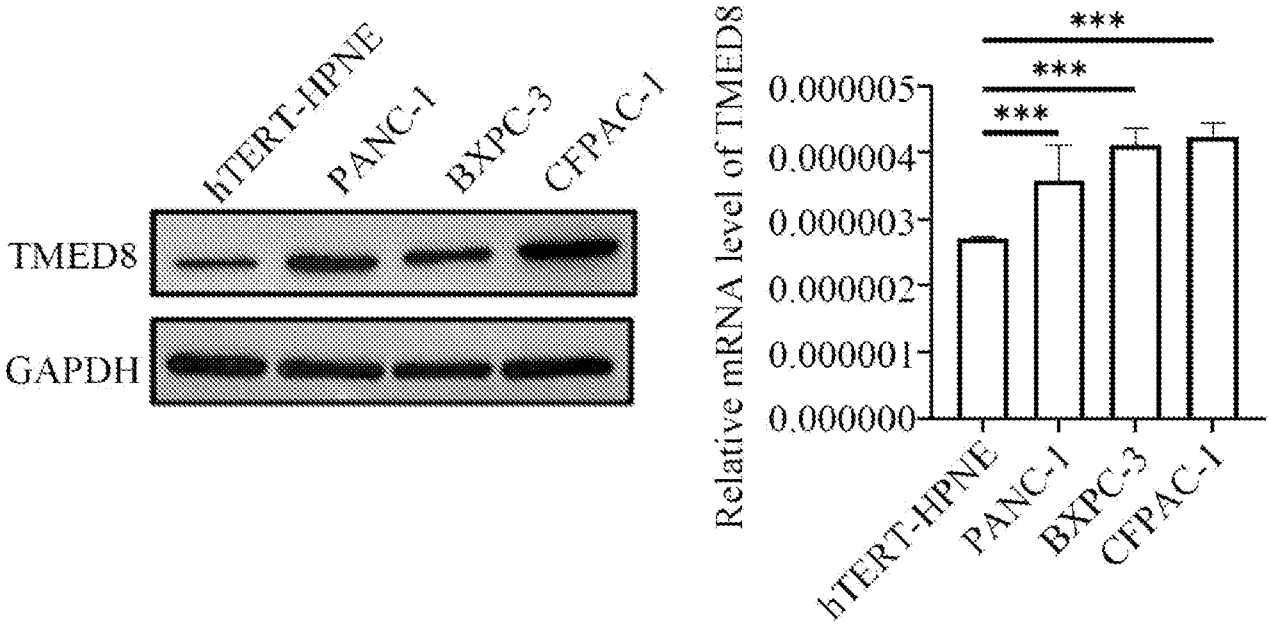

hTERT-HPNE
PANC-1
BXPC-3
CFPAC-1
TMED8
GAPDH
Relative mRNA level of TMED8
0.000005
0.000004
0.000003
0.000002
0.000001
0.000000
***
***
***
hTERT-HPNE
PANC-1
BXPC-3
CFPAC-1

FIG. 4

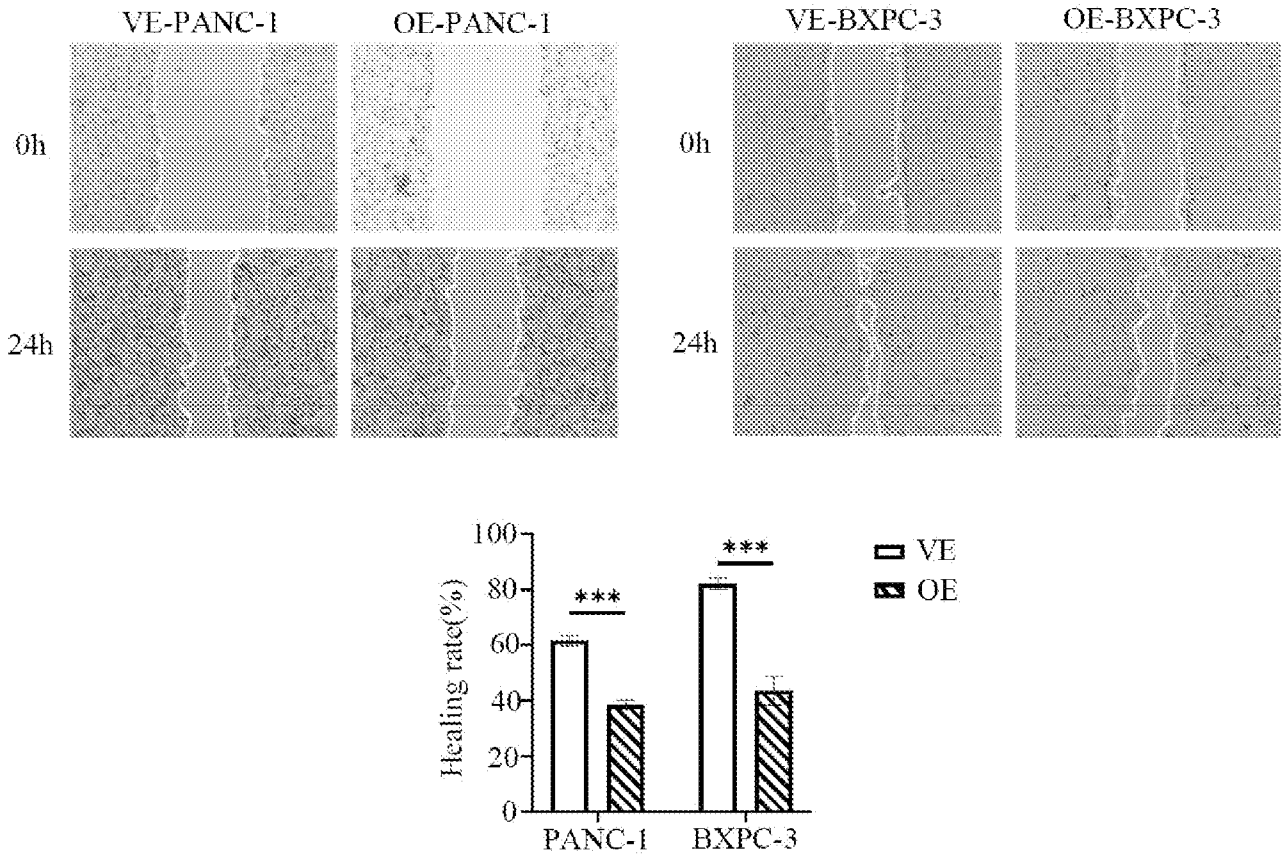

VE-PANC-1
OE-PANC-1
VE-BXPC-3
OE-BXPC-3
0h
24h
0h
24h
100
80
60
40
20
0
Healing rate(%)
***
***
PANC-1
BXPC-3
VE
OE

FIG. 5

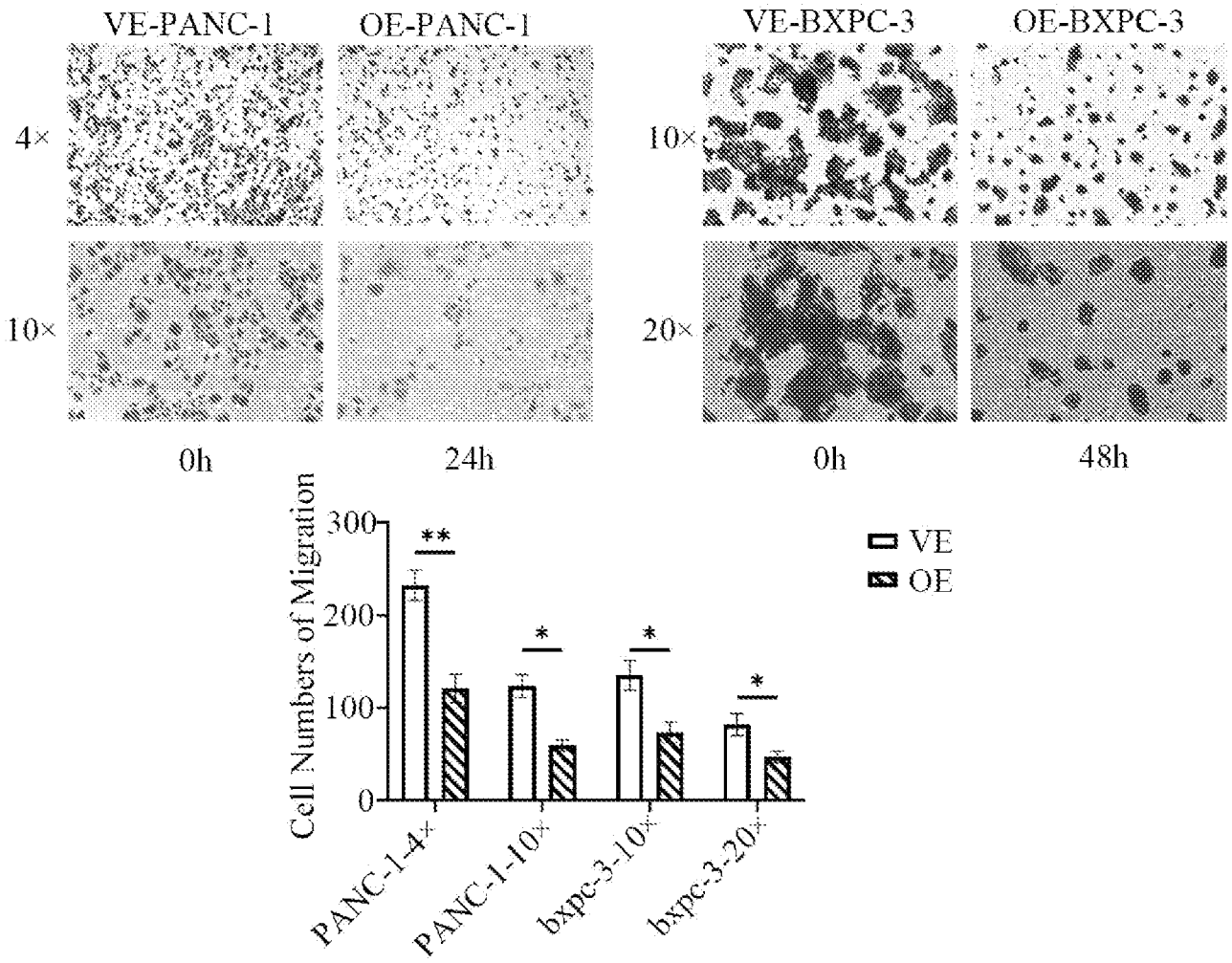

VE-PANC-1
OE-PANC-1
VE-BXPC-3
OE-BXPC-3
4×
10×
10×
20×
0h
24h
0h
48h
Cell Numbers of Migration
300
200
100
0
**
*
*
*
VE
OE
PANC-1-4×
PANC-1-10×
bxpc-3-10×
bxpc-3-20×

FIG. 6

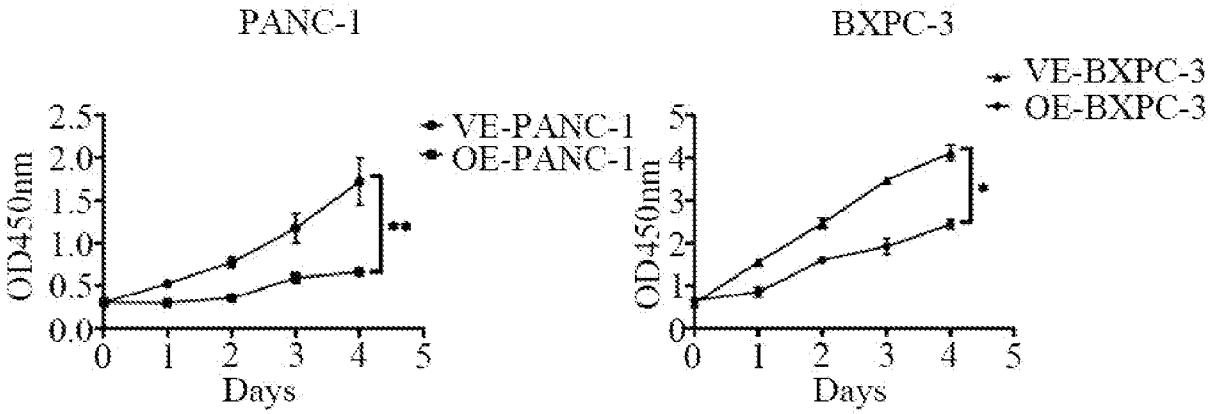

PANC-1
BXPC-3
OD450nm
Days
VE-PANC-1
OE-PANC-1
VE-BXPC-3
OE-BXPC-3
**
*

FIG. 7

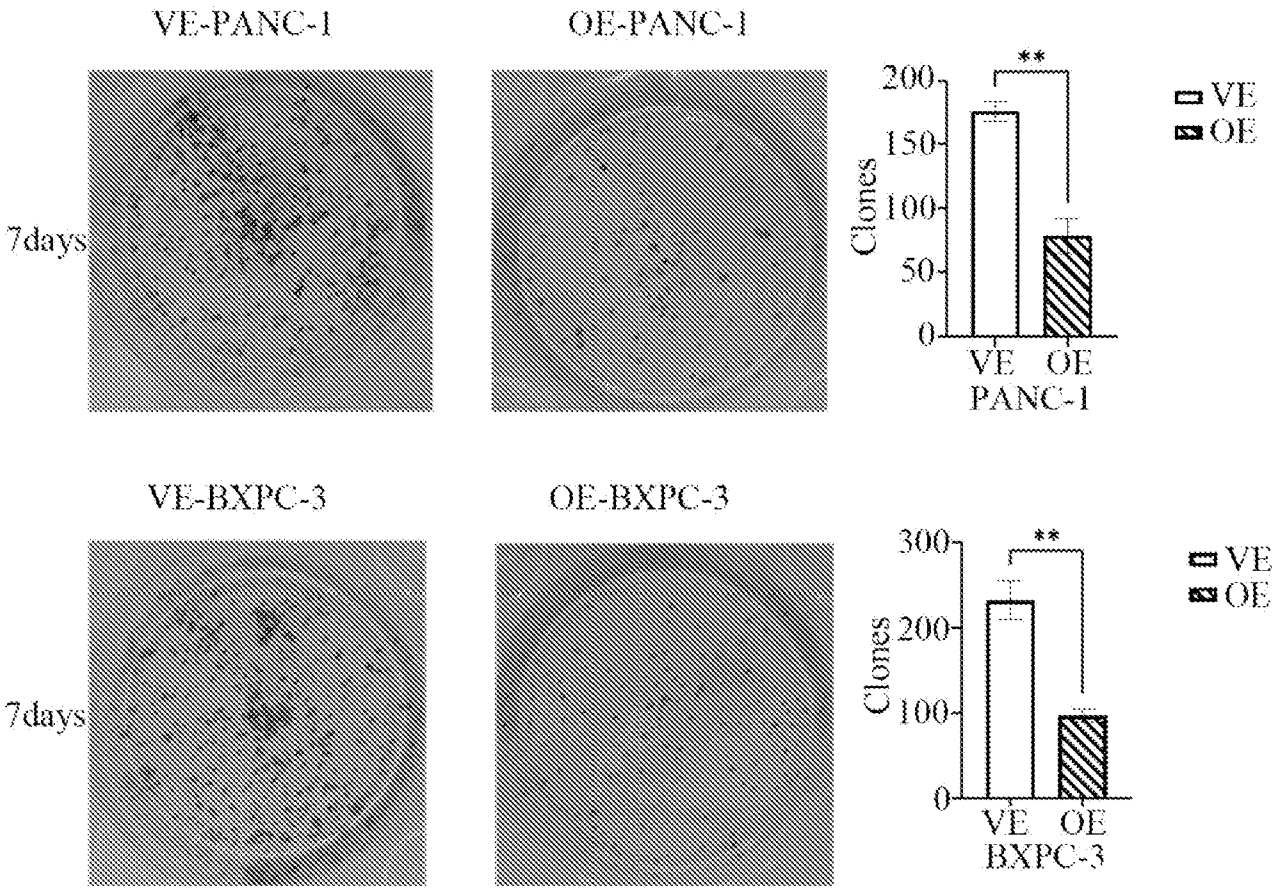

VE-PANC-1
OE-PANC-1
7days
200
150
100
50
0
Clones
**
VE
OE
VE OE
PANC-1
VE-BXPC-3
OE-BXPC-3
7days
300
200
100
0
Clones
**
VE
OE
VE OE
BXPC-3

FIG. 8

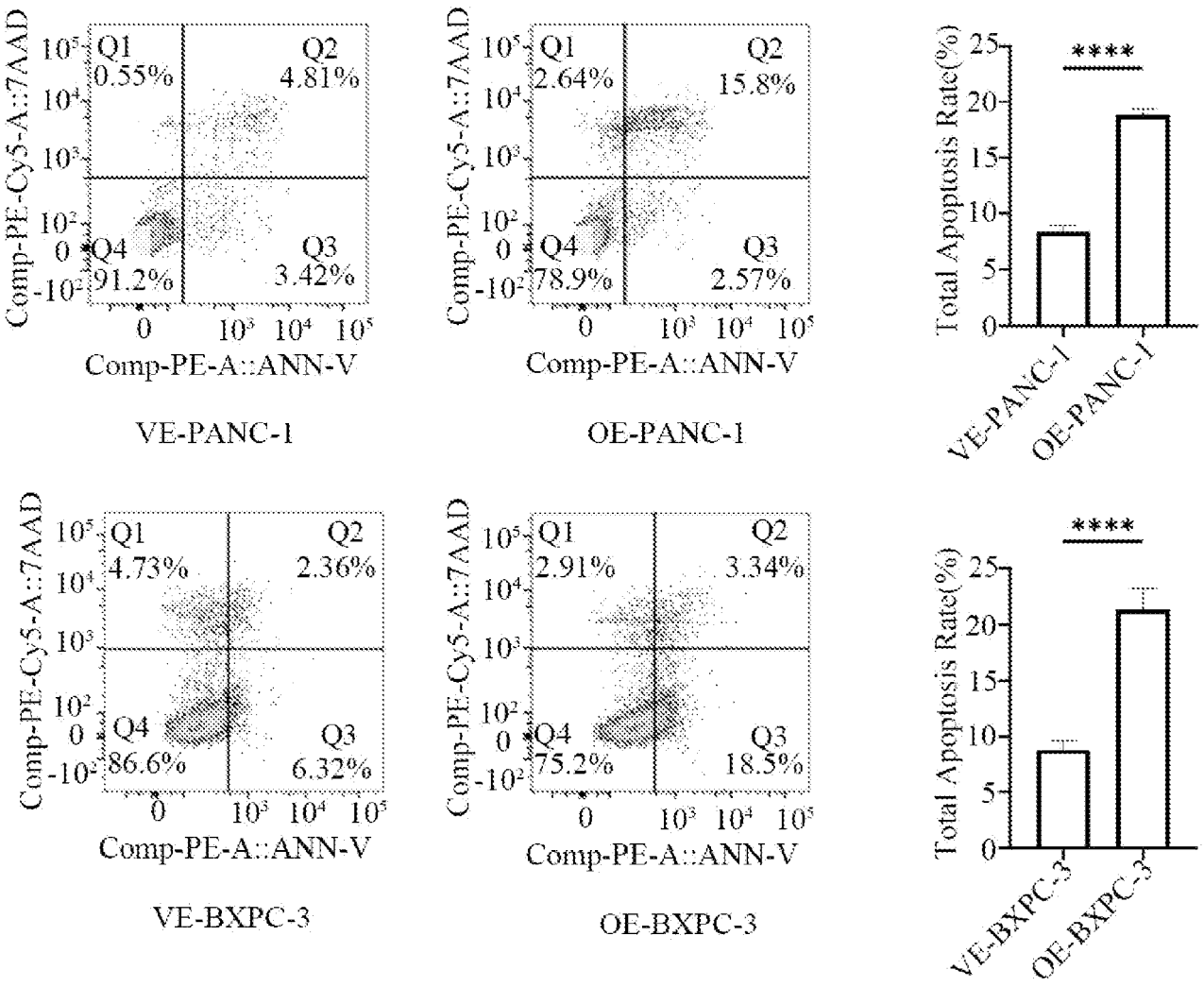

Comp-PE-Cy5-A::7AAD
Comp-PE-A::ANN-V
Q1
0.55%
Q2
4.81%
Q4
91.2%
Q3
3.42%
VE-PANC-1
Q1
2.64%
Q2
15.8%
Q4
78.9%
Q3
2.57%
OE-PANC-1
Total Apoptosis Rate(%)
****
VE-PANC-1
OE-PANC-1
Q1
4.73%
Q2
2.36%
Q4
86.6%
Q3
6.32%
VE-BXPC-3
Q1
2.91%
Q2
3.34%
Q4
75.2%
Q3
18.5%
OE-BXPC-3
Total Apoptosis Rate(%)
****
VE-BXPC-3
OE-BXPC-3

FIG. 9

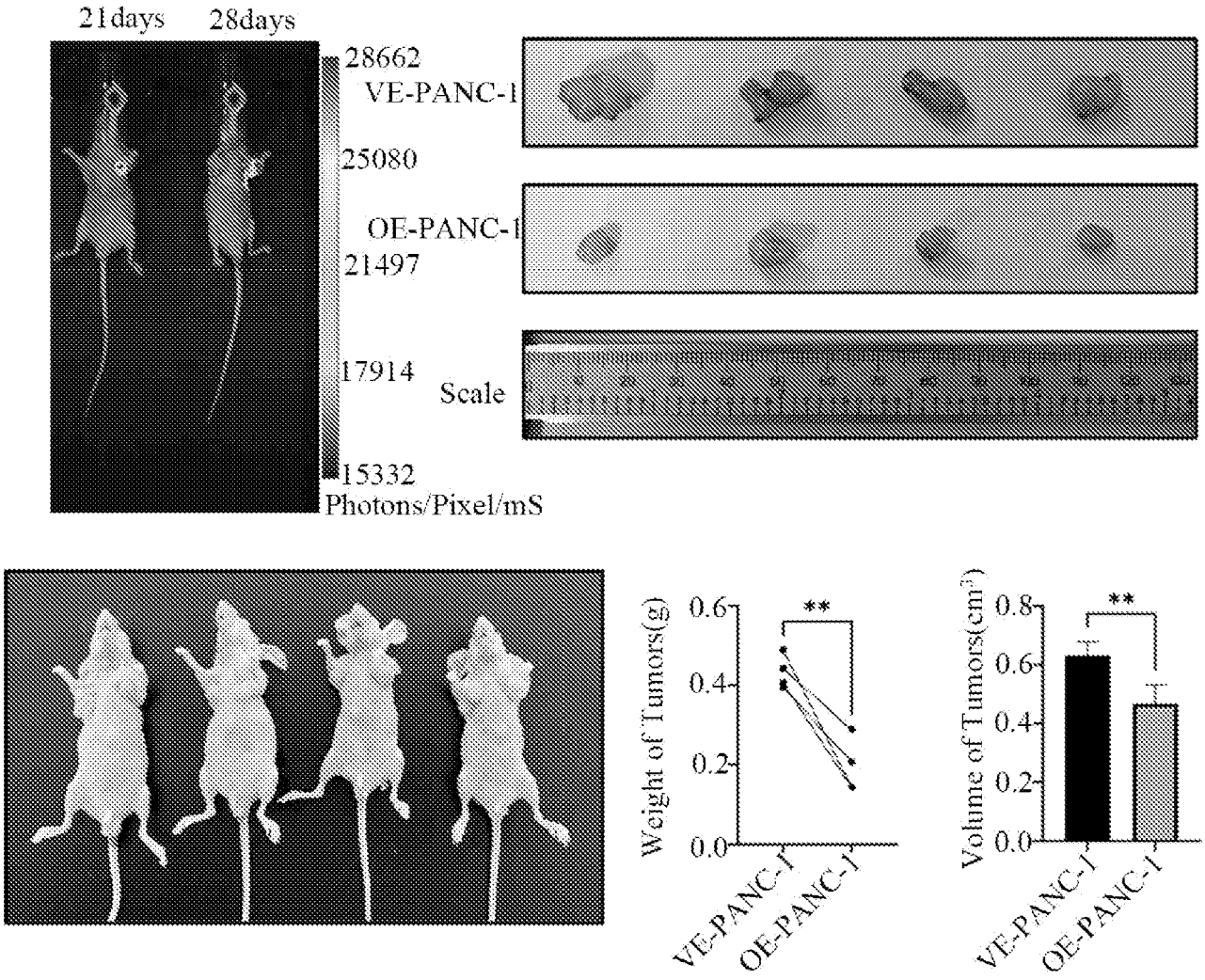

21days
28days
28662
VE-PANC-1
25080
OE-PANC-1
21497
17914
Scale
15332
Photons/Pixel/mS
Weight of Tumors(g)
0.6
0.4
0.2
0.0
**
VE-PANC-1
OE-PANC-1
Volume of Tumors(cm3)
0.8
0.6
0.4
0.2
0.0
**
VE-PANC-1
OE-PANC-1

FIG. 10

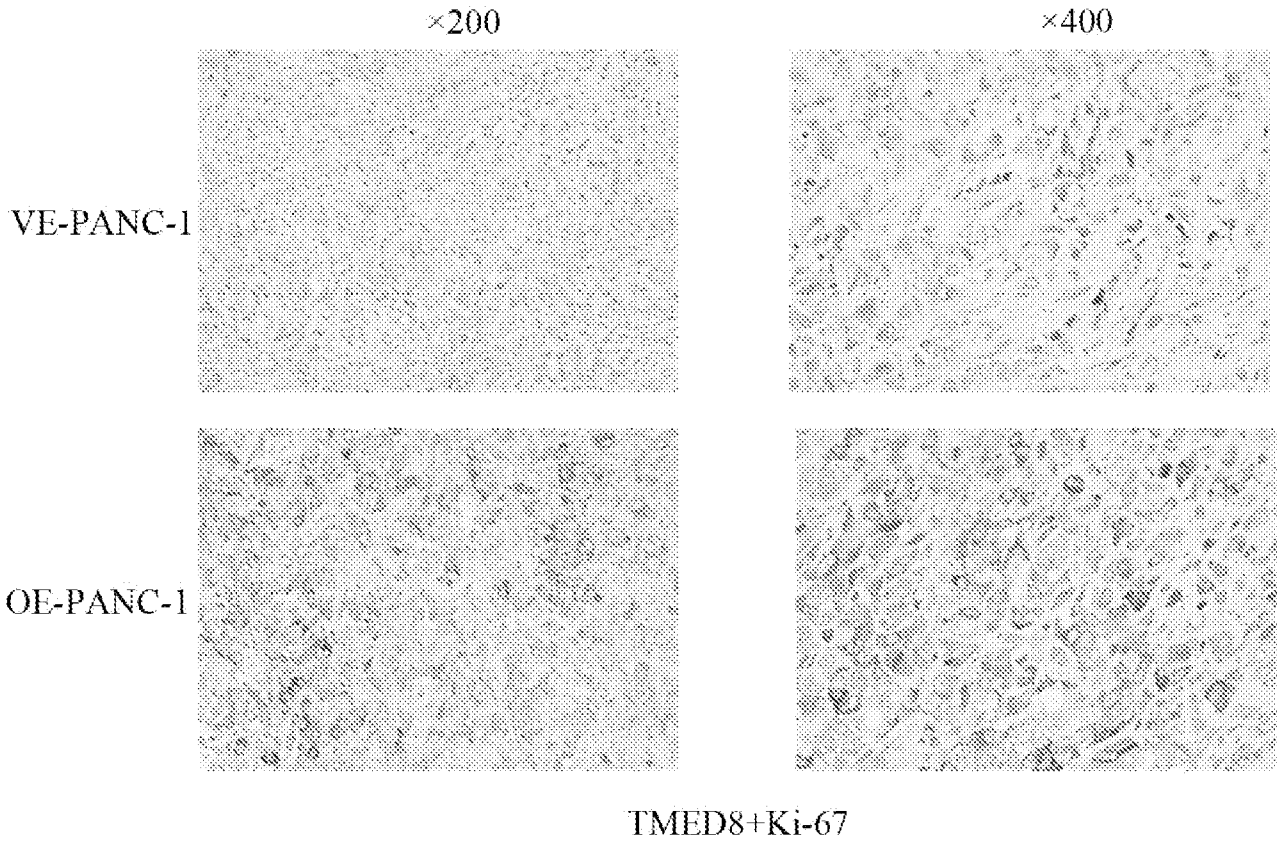


FIG. 11

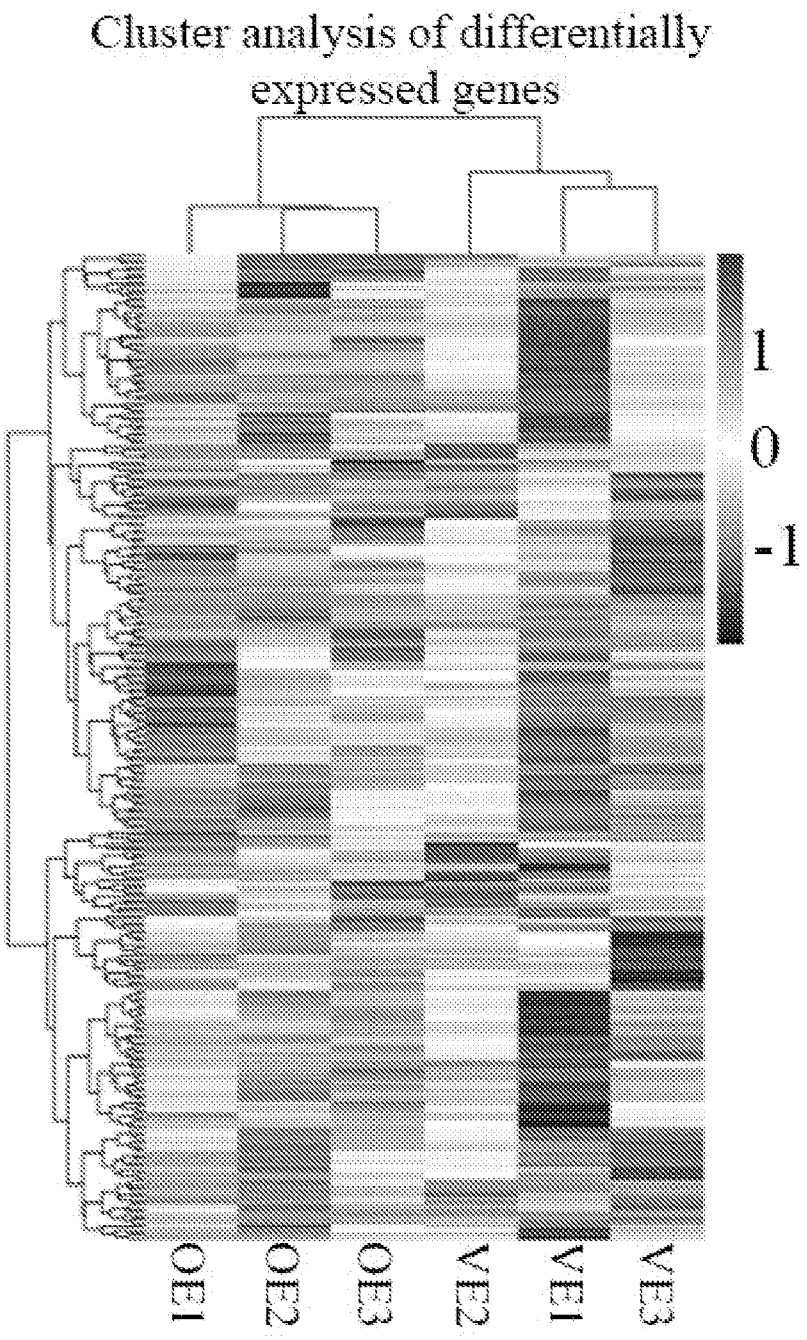

Cluster analysis of differentially expressed genes
1
0
-1
OE1
OE2
OE3
VE2
VE1
VE3

FIG. 12

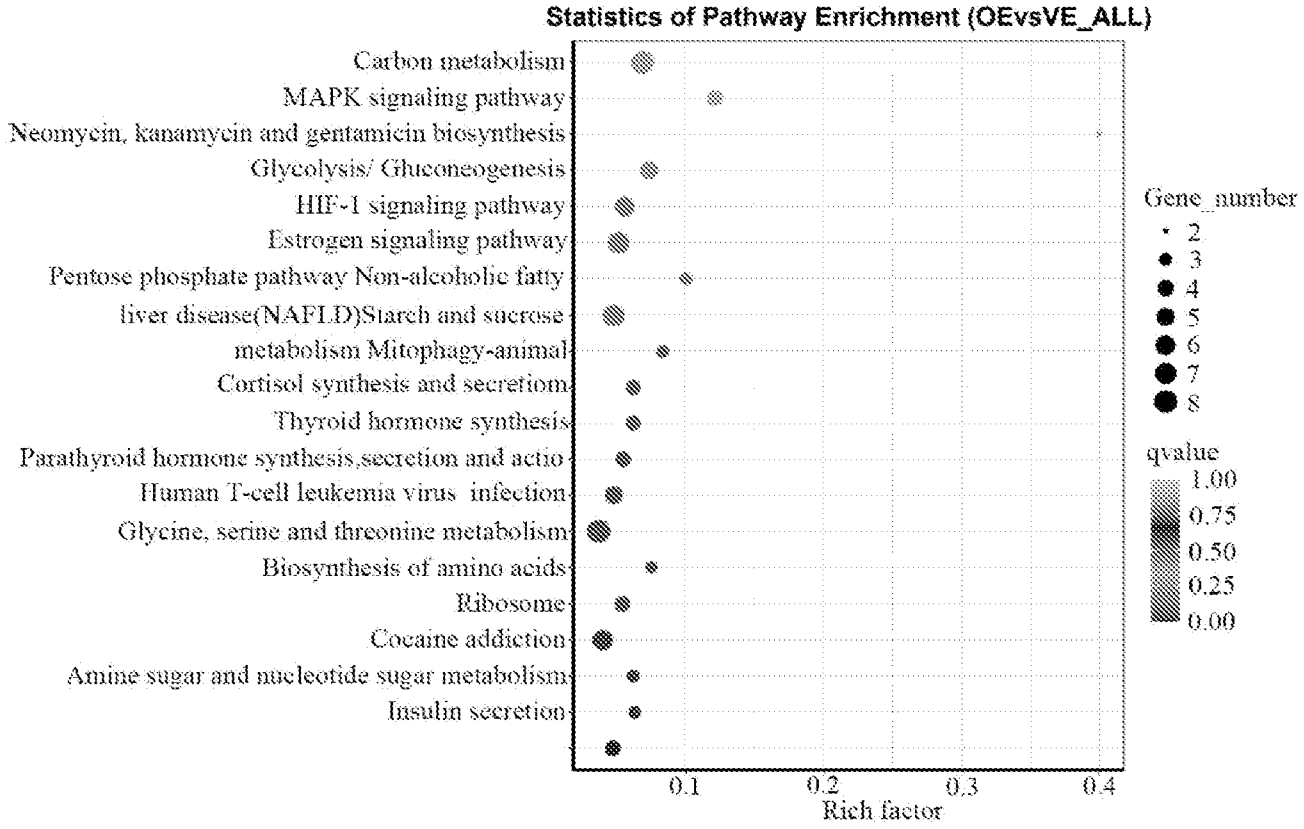

Statistics of Pathway Enrichment (OEvsVE_ALL)
Carbon metabolism
MAPK signaling pathway
Neomycin, kanamycin and gentamicin biosynthesis
Glycolysis/ Gluconeogenesis
HIF-1 signaling pathway
Estrogen signaling pathway
Pentose phosphate pathway Non-alcoholic fatty
liver disease(NAFLD)Starch and sucrose
metabolism Mitophagy-animal
Cortisol synthesis and secretion
Thyroid hormone synthesis
Parathyroid hormone synthesis,secretion and actio
Human T-cell leukemia virus infection
Glycine, serine and threonine metabolism
Biosynthesis of amino acids
Ribosome
Cocaine addiction
Amine sugar and nucleotide sugar metabolism
Insulin secretion
0.1
0.2
0.3
0.4
Rich factor
Gene_number
2
3
4
5
6
7
8
qvalue
1.00
0.75
0.50
0.25
0.00

FIG. 13

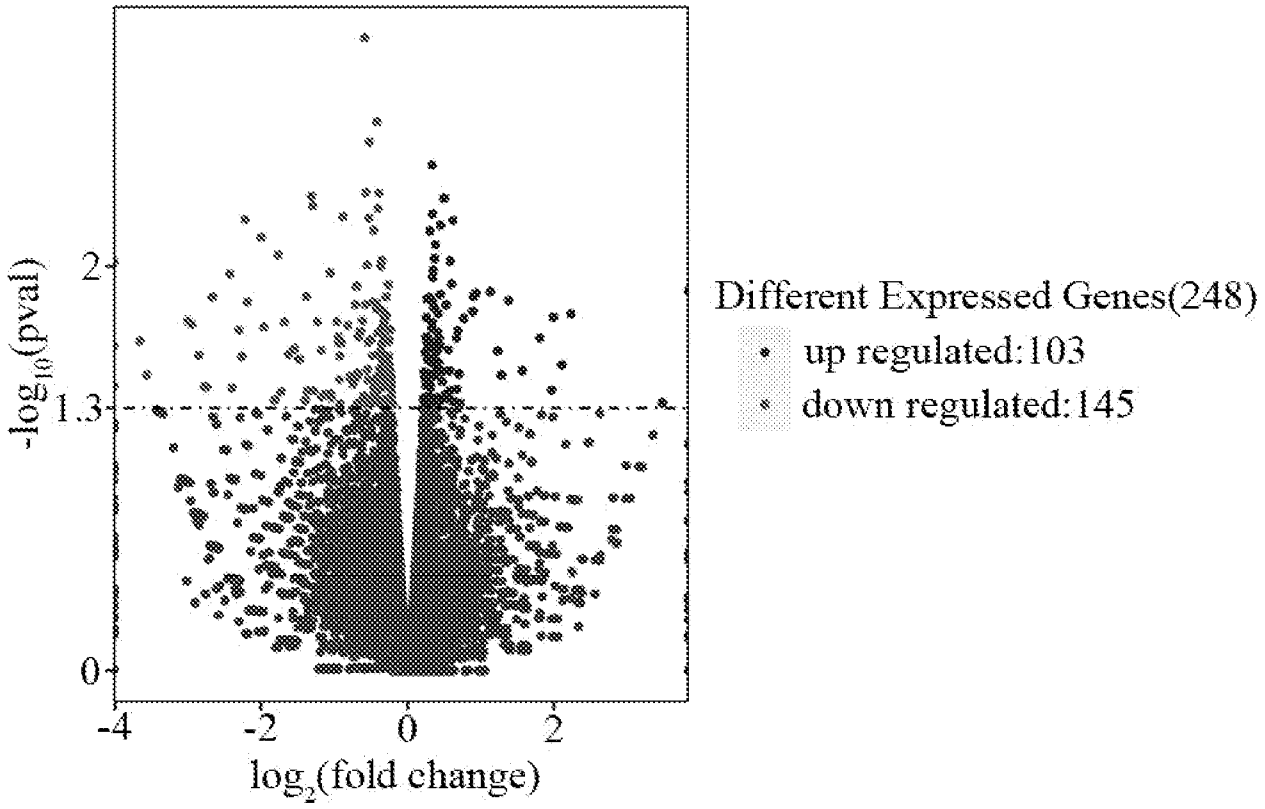

-log10(pval)
2
1.3
0
-4
-2
0
2
log2(fold change)
Different Expressed Genes(248)
up regulated:103
down regulated:145

FIG. 14

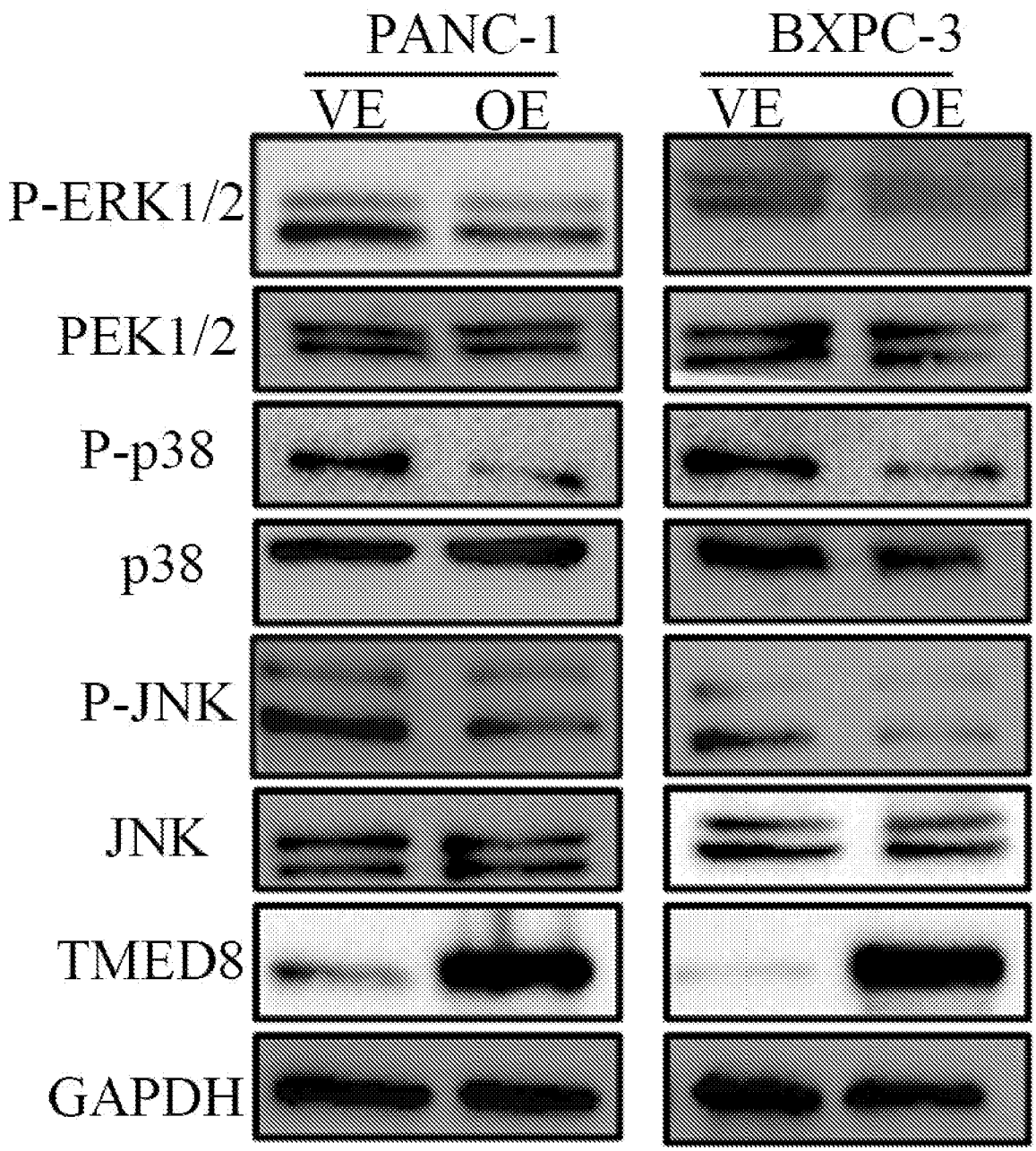
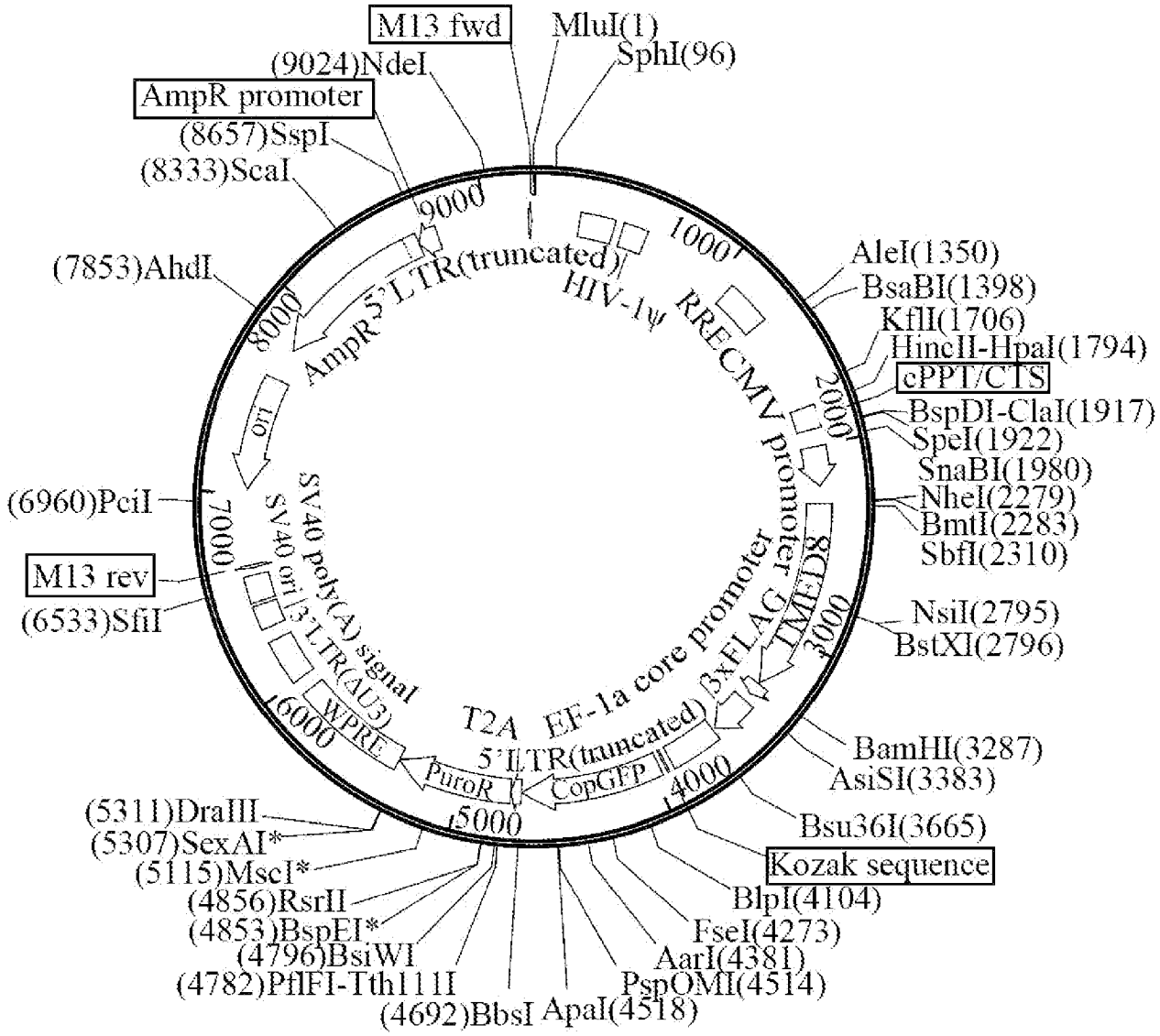

PANC-1
BXPC-3
VE
OE
VE
OE
P-ERK1/2
PEK1/2
P-p38
p38
P-JNK
JNK
TMED8
GAPDH

FIG. 15



pLV3-CMV TMED8(human)-3xFLAG-EF1a-CopGFP-Puro
9244bp

FIG. 16

USE OF *TMED8* GENE IN PREPARATION OF DRUG FOR TUMOR TREATMENT AND DIAGNOSTIC KIT

TECHNICAL FIELD

[0001] The present disclosure belongs to the technical field of precision tumor medicine, and specifically relates to use of a transmembrane emp24 domain 8 (*TMED8*) gene in preparation of a drug for tumor treatment and a diagnostic kit.

BACKGROUND

[0002] Pancreatic cancer (PC), as an extremely-common and highly-malignant tumor with difficult early diagnosis, easy metastasis, rapid progression, and extremely poor prognosis, is a leading cause of cancer death worldwide.Inherited genetic factors, although not directly modifiable, are an important component of pancreatic cancer risk, and include pathogenic variants in inherited cancer genes, genes associated with hereditary pancreatitis, and common variants identified in genome-wide association study (GWAS). Identifying genetic changes in cancer not only provides insight into the cause of this cancer but also provides the opportunity to guide early detection strategies. The pancreatic cancer has no obvious symptoms in its early stages, making it difficult to detect and diagnose in time. Tumor markers that are currently commonly used in clinical practice, such as CA199 and CEA, have poor sensitivity and specificity for the early diagnosis of pancreatic cancer. Once diagnosed, most patients with pancreatic cancer are already in the intermediate and advanced stages. In addition to radical surgical resection, adjuvant radiotherapy and chemotherapy and other methods show extremely limited improvement in prognosis. Therefore, it is of great significance to explore and discover early markers and effective therapeutic targets with greater sensitivity and specificity.

[0003] Transmembrane emp24 domain family (TMED family) consists of type I single-channel transmembrane proteins, is found in all eukaryotic organisms, and plays a role in human development and disease that cannot be ignored. The TMED is an important transporter in the secretory pathway. Once a protein transport pathway controlled by TMED is abnormal, there may be occurrence of various diseases including congenital malformations, diabetes, and cancer. Previous studies have shown that abnormal expression of TMED proteins is generally closely related to liver diseases, pancreatic diseases, and immune system disorders. The TMED proteins may form a secretory loop during the development of diseases, maintain a normal shape of cells and intracellular organelles, circulate and transport a variety of important cargo components between organelles, and achieve the communication between organelles. As a result, the TMED proteins continuously regulate a disease process, and especially play an important role in the proliferation

and differentiation of malignant tumor cells.

[0004] TMED8 (NCBI Gene: 283578), one of the important members in the TMED family, is located between bases 77,335,021 to 77,377,094 of chromosome 14, has a molecular weight of approximately 55 kDa, and can be expressed in both the nucleus and cytoplasm. There are currently no literature reports on a relationship between the TMED8 and human diseases and normal physiological functions.

SUMMARY

[0005] A purpose of the present disclosure is to provide use of a *TMED8* gene in preparation of a drug for tumor treatment and a diagnostic kit, as well as use of the *TMED8* gene in preparation of a drug for treating a tumor.

[0006] The present disclosure provides use of a reagent for detecting an expression level of a *TMED8* gene in preparation of a product for diagnosing a disease.

[0007] In one embodiment, the reagent includes a reagent for detecting an expression level of an mRNA or a protein of the *TMED8* gene.

[0008] In one embodiment, a method for detecting the expression level of the *TMED8* gene is at least one selected from the group consisting of immunohistochemistry, Western Blot, and real-time fluorescence quantitative PCR.

[0009] In one embodiment, the disease includes a tumor.

[0010] The present disclosure further provides a kit for diagnosing a tumor, including at least one of the following reagents: a reagent for detecting an expression level of a TMED8 protein using immunohistochemistry, a reagent for detecting the expression level of the TMED8 protein using Western Blot, and a primer pair for detecting an expression level of a *TMED8* gene and/or an mRNA using real-time fluorescence quantitative PCR.

[0011] In one embodiment, the tumor includes pancreatic cancer.

[0012] The present disclosure further provides use of a reagent for increasing an expression level of a *TMED8* gene in preparation of a product for treating a disease.

[0013] In one embodiment, the reagent is at least one selected from the group consisting of an agonist of the *TMED8* gene, an analog of the *TMED8* gene, and an agent for overexpressing the *TMED8* gene.

[0014] The present disclosure further provides a drug for treating a tumor, including an agent for increasing an expression level of an endogenous *TMED8* gene or an exogenous *TMED8* gene.

[0015] In one embodiment, the treating includes at least one of the following: inhibiting tumor cell migration, inhibiting tumor cell proliferation, promoting tumor cell apoptosis, and inhibiting mitogen-activated protein kinase (MAPK) signaling pathway activation.

[0016] Beneficial effects: in the present disclosure, expression of the *TMED8* gene in pancreatic cancer is detected using immunohistochemistry by preparing a pancreatic cancer tissue chip. The results have showed that the expression level of the *TMED8* gene in a pancreatic cancer tumor tissue is significantly higher than that in tissues adjacent to cancer. In addition, a stably-transfected cell line of pancreatic cancer overexpressing *TMED8* gene is established, and the influence of *TMED8* gene on the biological behavior of pancreatic cancer cells is explored through *in vivo* and *in vitro* experiments such as qRT-PCR, Western Blot, cell scratching, CCK8, Transwell, flow cytometry, and subcutaneous tumor formation in nude mice. It is found that up-regulating an expression level of the *TMED8* gene has significantly inhibited migration and proliferation of pancreatic cancer cells, and then promoted apoptosis of the pancreatic cancer cells; the *TMED8* gene is further found to be related to a MAPK signaling pathway. Accordingly, the *TMED8* gene may be used as an effective target for gene diagnosis and treatment of pancreatic cancer, and has an important application significance in the preparation of diagnostic kits and therapeutic drugs for precision medicine of the pancreatic cancer. The present disclosure explores an expression state in pancreatic cancer, an impact on the biological behavior of pancreatic cancer cells of the *TMED8* gene, and a possible mechanism of action that is closely related to the MAPK signaling pathway. The *TMED8* gene is expected to be used as a diagnostic kit as well as a therapeutic drug for pancreatic cancer, thus providing an effective support for early diagnosis and treatment of the pancreatic cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1 shows the expression of *TMED8* gene detected by immunohistochemistry (IHC) in pancreatic cancer and tissue chips adjacent to cancer; where representative sites are displayed: a to b represent the staining results of poorly-differentiated PC cancer and tissues adjacent to cancer; c to d represent the staining results of moderately-differentiated PC cancer and tissues adjacent to cancer; and e to f represent the staining results of highly-differentiated PC cancer and tissues adjacent to cancer;

[0018] FIG. 2 shows the expression of *TMED8* gene and the corresponding WB and PCR verification results;

[0019] FIG. 3 shows a comparison of the expression of *TMED8* gene in normal ductal epithelial cells and tumor cell lines of pancreatic cancer;

[0020] FIG. 4 shows verification for the efficiency of *TMED8* gene overexpression in stably-transfected cell lines of pancreatic cancer;

[0021] FIG. 5 shows an influence of overexpression of the *TMED8* gene on a migration ability of pancreatic cancer cells studied by cell scratching experiments;

[0022] FIG. 6 shows an influence of overexpression of the TMED8 gene on a migration ability of pancreatic cancer cells studied by Transwell experiments;

[0023] FIG. 7 shows an influence of overexpression of the TMED8 gene on a proliferation ability of pancreatic cancer cells studied by CCK8 experiments;

[0024] FIG. 8 shows an influence of overexpression of the *TMED8* gene on a proliferation ability of pancreatic cancer cells studied by colony formation experiments;

[0025] FIG. 9 shows an influence of overexpression of the *TMED8* gene on an apoptotic ability of pancreatic cancer cells detected by flow cytometry;

[0026] FIG. 10 shows an influence of overexpression of the *TMED8* gene on subcutaneous tumor formation of pancreatic cancer cells in nude mice;

[0027] FIG. 11 shows an influence of overexpression of the *TMED8* gene as illustrated by immunohistochemical (IHC) staining for *TMED8* and the proliferation marker *Ki67*, in tissue samples from subcutaneous tumor induced by pancreatic cancer cells in nude mice.

[0028] FIG 12 shows a heatmap from transcriptome sequencing of a pancreatic cancer cell line overexpressing *TMED8*.

[0029] FIG. 13 shows a volcano plot depicting the number of differentially expressed molecules identified from transcriptome sequencing of a pancreatic cancer cell line overexpressing *TMED8*.

[0030] FIG. 14 shows results of KEGG enrichment analysis for the differentially expressed molecules following *TMED8* overexpression.

[0031] FIG. 15 shows an influence of overexpression of the *TMED8* gene on MAPK-related molecules; and

[0032] FIG. 16 shows a construction map of an overexpression plasmid pLV3-CMV-TMED8(human)-3×FLAG-EF1a-CopGFP-Puro of the *TMED8* gene.

DETAILED DESCRIPTION OF THE EMBODIMENTS

[0033] The present disclosure provides use of a reagent for detecting an expression level of a *TMED8* gene in preparation of a product for diagnosing a disease.

[0034] In the examples of the present disclosure, a pancreatic cancer tissue chip is prepared based on a biological sample bank, and expression of the *TMED8* gene in pancreatic cancer is detected using immunohistochemistry by preparing a pancreatic cancer tissue chip. The results have showed that the expression level of the *TMED8* gene in a pancreatic cancer tumor tissue is significantly higher than that in tissues adjacent to cancer. Moreover, according to statistics, there is better prognosis of patients in a *TMED8* gene high expression group, such that the *TMED8* gene is used as a biomarker for the diagnosis of pancreatic cancer.

[0035] In the present disclosure, the reagent preferably includes a reagent for detecting an

expression level of an mRNA or a protein of the *TMED8* gene, and a detection method preferably includes at least one of the following: immunohistochemistry, Western Blot, and real-time fluorescence quantitative PCR. The disease preferably includes a tumor, more preferably pancreatic cancer.

[0036] The present disclosure further provides a kit for diagnosing a tumor, including at least one of the following reagents: a reagent for detecting an expression level of a TMED8 protein using immunohistochemistry, a reagent for detecting the expression level of the TMED8 protein using Western Blot, and a primer pair for detecting an mRNA expression level of the *TMED8* gene by real-time fluorescence quantitative PCR. The primer sequences of the *TMED8* gene and an internal reference 18S are shown in Table 1.

[0037] Table 1 Primer sequences

[0038]

Gene	Sequence	SEQ ID NO:
TMED8	F:CCACCTCTGATGGCTCCT	1
	R:CTCACCACGCTTCACCAC	2
18S	F:GGAGCCTGCGGCTTAATTTG	3
	R:CCACCCACGGAATCGAGAAA	4

[0039] In the present invention, the tumor preferably includes pancreatic cancer.

[0040] The present disclosure further provides use of a reagent for increasing an expression level of a *TMED8* gene in preparation of a product for treating a disease.

[0041] In the examples of the present disclosure, a stably-transfected cell line of pancreatic cancer overexpressing *TMED8* gene is established, and the influence of *TMED8* gene on the biological behavior of pancreatic cancer cells is explored through *in vivo* and *in vitro* experiments such as qRT-PCR, Western Blot, cell scratching, CCK8, Transwell, flow cytometry, and subcutaneous tumor formation in nude mice. It is found that up-regulating an expression level of the *TMED8* gene has significantly inhibited migration and proliferation of pancreatic cancer cells, and then promoted apoptosis of the pancreatic cancer cells; the *TMED8* gene is further found to be related to a MAPK signaling pathway. Therefore, the *TMED8* gene can be used as an effective target for gene treatment of the pancreatic cancer.

[0042] In the present disclosure, the reagent is preferably at least one selected from the group consisting of an agonist of the *TMED8* gene, an analog of the *TMED8* gene, and an agent for overexpressing the *TMED8* gene.

[0043] In the present disclosure, a method for overexpressing the *TMED8* gene preferably includes constructing an overexpression plasmid of the *TMED8* gene, pLV3-CMV-TMED8(human)-3×FLAG-EF1a-CopGFP-Puro, and the plasmid has a sequence

shown in SEQ ID NO: 5.

[0044] The present disclosure further provides a drug for treating a tumor, including an agent for increasing an expression level of an endogenous *TMED8* gene or an exogenous *TMED8* gene.

[0045] In the present disclosure, a reagent for increasing an expression level of an endogenous *TMED8* gene is preferably the same as the reagent for overexpressing the *TMED8* gene, and will not be repeated here. The exogenous *TMED8* gene is preferably the same as the agonist of the *TMED8* gene and the analog of the *TMED8* gene, and will not be repeated here. The treating includes preferably at least one of the following: inhibiting tumor cell migration, inhibiting tumor cell proliferation, promoting tumor cell apoptosis, and inhibiting MAPK signaling pathway activation.

[0046] To further illustrate the present disclosure, the use of the *TMED8* gene in preparation of the drug and the diagnostic kit for pancreatic cancer provided by the present disclosure are described in detail below in connection with examples, but these examples should not be construed as limiting the claimed scope of the present disclosure.

[0047] In the examples of the present disclosure, the reagents and instruments used are common reagents and instruments in the field unless otherwise specified, and can be purchased from reagent stores:

[0048] 1. The main reagents in the examples of the present disclosure include the following:

[0049] IHC detection kit (Servicebio, China); 4% paraformaldehyde fixation solution (Servicebio, China); PBS buffer (Servicebio, China); bovine serum albumin (BSA) powder (Solarbio Life Sciences, China); Triton X-100 (Solarbio Life Sciences, China); xylene (Sinopharm Group, China); hematoxylin staining solution (Beyotime, China); hematoxylin differentiation solution (Beyotime, China); absolute ethanol (Sinopharm Group, China); sodium citrate antigen retrieval solution (Beyotime, China); rabbit-derived anti-TMED8 antibody (Invitrogen, USA); rabbit-derived anti-Ki67 (Absin, China); HRP-labeled goat anti-rabbit secondary antibody (Abcam, USA); 95% ethanol (Sinopharm Group, China); DMEM basal medium (Cytiva, China); IMDM basal medium (Cytiva, China); RPMI basal medium (Cytiva, China); PBS basal buffer (Servicebio, China); fetal bovine serum (FBS) (Gibco, United States); serum-free cell cryopreservation medium (NCM Biotech, China); trypsin digestion solution containing EDTA (NCM Biotech, China); penicillin-streptomycin solution (NCM Biotech, China); tetracycline hydrochloride solution (Shanghai Sangon, China); puromycin (Sangon, USA); cell culture dish (Nest, China); Lipofectamine 2000 (Invitrogen, USA); 6-well plate, 96-well plate, 24-well plate (Nest, China); 8 μm transparent PET membrane for cell culture chamber (BD Falcon, USA); 1.5 mL, 4 mL, 10 mL centrifuge tubes, cell cryopreservation tube (Biosharp, USA); Trizol (Novizan, China); cell scraper (Nest, China); DEPC water (Shanghai Sangon, China); LC eight-tube strip (Roche, Switzerland);

enzyme-free pipette tip, centrifuge tube (Biosharp, China); absolute ethanol (Sinopharm Group, China); isopropyl alcohol (Sinopharm Group, China); chloroform (Sinopharm Group, China); (1) RIPA powerful lysis buffer (Beyotime, China); protease inhibitor CM (Amresco, USA); phosphatase inhibitor PI (Amresco, USA); BCA protein analysis kit (Beyotime, China); protein 4× Loading Buffer (Novizan, China); Marker standard protein (Novizan, China); PVDF membrane (Sigma, USA); tetramethylethylenediamine (TEMED) (Sigma, USA); 0×electrophoresis solution pre-made powder (Servicebio, Wuhan); 10× transfer membrane solution pre-made powder (Servicebio, Wuhan); 10× TBST pre-made powder (Servicebio, Wuhan); sodium chloride (NaCl) (Sinopharm Group, China); isopropyl alcohol (Sinopharm Group, China); ammonium persulfate (AP) (Sigma, USA); glycine (Sinopharm Group, China) China); sodium dodecyl sulfate (SDS) (Sinopharm Group, China); Tris (Sinopharm Group, China); methanol (Sinopharm Group, China); skim milk powder (Yili Dairy, China); ultra-sensitive ECL chemiluminescence kit (NCM Biotech, China); 30% polyacrylamide solution (Servicebio, China); Tween (Sinopharm Group, China); mouse anti-human GAPDH antibody (Proteintech, China); rabbit anti-human TMED8 antibody (Invitrogen, USA); rabbit anti-human ERK1/2 antibody (CST, USA); rabbit anti-human ERK phosphorylated antibody (CST, USA); rabbit anti-human P38 antibody (CST, USA); rabbit anti-human P38 phosphorylated antibody (CST, USA); rabbit anti-human JNK antibody (CST, USA); rabbit anti-human JNK phosphorylated antibody (CST, USA); rabbit anti-human Casepase3 Antibodies (Santa, Japan); rabbit anti-human CD63 antibody (Abcam, USA); Rabbit anti-human CD81 antibody (Abcam, USA); rabbit anti-human CD9 antibody (Abcam, USA); mouse anti-human Ki-67 antibody (Wuhan Sanying, China); HRP-labeled goat anti-rabbit IgG antibody (Invitrogen, USA); HRP-labeled goat anti-mouse IgG antibody (Invitrogen, USA); CCK-8 kit (Beyotime, China); cell cycle analysis kit (Formax Biotech, China); cell apoptosis detection kit: AnnexinV-PE/7-AAD double-staining (KGI Biotech, China); and crystal violet staining solution (Beyotime, China).

[0050] 2. The main instruments in the examples of the present disclosure include the following:

[0051] tissue chip system (UT06, Unitma, South Korea); inverted microscope (Leica, Germany); chemiluminescent gel imaging system (Bio-Rad, USA); fully automatic microplate reader (Bio-Rad, USA); electrophoresis system (Bio-Rad, USA); and cell culture incubator (Thermo Fisher, USA).

[0052] Example 1

[0053] Between 2020 and 2023, 79 formalin-fixed paraffin-embedded (FFPE) pancreatic cancer tissue samples, including cancer and tissues adjacent to cancer, were obtained from the Clinical Biobank of Affiliated Hospital of Nantong University. No cancer patients received any treatment, including radiotherapy, chemotherapy, or immunotherapy, and clinical case data were detailed and complete. This study protocol was approved by the Human Research Ethics Committee of Affiliated

Hospital of Nantong University, China.

[0054] 1. Production of tissue chips:

[0055] (1) According to the microscopic observation results of HE sections, the representative cancer nest areas in the wax block were marked.

[0056] (2) The paraffin wax and beeswax were mixed at a ratio of 1:1 to form a TMA blank receptor wax block. A 10×7-dot tissue array was designed on the wax block, and then holes were punched with a tissue chip instrument to make a TMA blank wax block.

[0057] (3) The most representative cancer nest area was selected at the marked point on the donor wax block, and a tissue block with a diameter of 2 mm was collected, with 1 core from each case.

[0058] (4) The tissue cores were transferred to the holes of the recipient wax block, with a certain distance between each tissue core, and the corresponding tissues adjacent to cancer were selected as a control.

[0059] (5) The tissue array block was fused by heating in a 55°C incubator for 10 min, the wax block was taken out before it was about to melt, and allowed to cool to room temperature, such that the receptor wax block and the donor tissue were integrated.

[0060] (6) Before slicing, the tissue chip was pre-cooled at 4°C for about 4 h, and then the tissue array block was corrected with a fully automatic tissue slicer at 20 mm/revolution until all tissues were complete.

[0061] (7) The tissue array block was sliced with a microtome, obtained continuous slices were floated in cold water to allow them to unfold naturally, and then the slices were transferred to warm water at 45°C to unfold for about 2 min. After unfolding, the slices were spread on a slide that had been treated to prevent detachment and allowed to air-dry.

[0062] (8) The slices were baked in a high-temperature oven at 60°C for 3 min, and then baked at 58°C for 18 h.

[0063] (9) The prepared tissue chips were stored in a sectioning box at -20°C for later use.

[0064] 2. Immunohistochemistry

[0065] (1) The tissue chip was baked in a constant-temperature oven at 60°C for about 40 min until the paraffin was melted.

[0066] (2) The chip was soaked in xylene I, xylene II, 100% ethanol I, and 100% ethanol II for 5 min each, and then soaked in 95% ethanol, 90% ethanol, 80% ethanol, and 70% ethanol for 2 min each, and then rinsed with water.

[0067] (3) An antigen retrieval solution was placed in an immunohistochemistry box in advance, preheated to 95°C using a water bath, then the chip was placed in the box to allow treatment at 95°C for 20 min for antigen retrieval, naturally cooled to room temperature, and then rinsed with PBS 3 times, 5 min each time.

[0068] (4) A boundary line was drawn with an immunohistochemistry pen to obtain a circle around the tissues on the chip, the inside of the circle was filled with 3% H_2O_2, and the tissues were incubated for 10 min, and then washed 3 times with PBS for 5 min each time.

[0069] (5) A primary antibody (rabbit anti-human TMED8 antibody) was added dropwise and incubated overnight at 4°C.

[0070] (6) Rewarming was conducted at room temperature for 1 h to remove the primary antibody, the tissues were rinsed with PBS 3 times for 5 min each time, a polymer enhancer was added dropwise, and incubated at room temperature for 20 min; and the tissues were washed with PBS 3 times for 5 min each time.

[0071] (7) A secondary antibody (enzyme-labeled anti-mouse/rabbit IgG polymer) was added dropwise, incubated at room temperature for 30 min, and then wash with PBS 3 times for 5 min each time.

[0072] (8) A freshly prepared DAB reagent was added dropwise, and after color development, the chip was rinsed with tap water for 5 min to terminate the color development.

[0073] (9) The chip was subjected to hematoxylin staining for 2 min, and immediately rinsed slowly with running water, then soaked in 0.5% HCl ethanol solution for 2 min, and rinsed with running water for 15 min.

[0074] (10) The chip was soaked in 80% ethanol, 90% ethanol, 95% ethanol, 100% ethanol I, 100% ethanol II, and xylene for 1 min each, and air-dried naturally in a fume hood.

[0075] (11) The tissue was sealed with neutral gum.

[0076] 3. Determination of results:

[0077] According to a degree of staining of cells in the tissues, the positive intensity was divided into 3 levels. No positive coloring was grade 0 (negative), light yellow was grade 1 (weak positive), brown was grade 2 (moderate positive), and tan was grade 3 (strong positive). Cell positivity rate = number of positive cells/total number of cells, which was divided into 4 levels; the rates of levels 0 to 4 are 0% to 5%, 6% to 25%, 26% to 50%, 51% to 75%, and >75%. The chip results were scored by 3 pathologists based on independent readings and then divided into four groups according to the quartiles of the scores: "-, +, ++, and +++" for statistics and analysis.

[0078] The results of immunohistochemical staining of 79 cases of pancreatic cancer tumors and slices for tissues adjacent to cancer were shown in FIG. 1. The positive TMED8 protein was mainly expressed in the cytoplasm of tumor cells and the interstitium of cancer tissues in brown-yellow color, while showing low expression or no expression in most tissues adjacent to cancer. Statistical results showed that the *TMED8* gene was indeed highly expressed in pancreatic cancer tissue chips $^*P<0.05$.

[0079] 8 pairs of cancer tissues and tissues adjacent to cancer from pancreatic cancer patients were

selected for WB verification. The results were shown in FIG. 2, and consistent with the above data.

[0080] Example 2

[0081] 1. Cell transfection

[0082] (1) Transformation and extraction of plasmid: a dry powder of an overexpression plasmid pLV3-CMV-TMED8(human)-3×FLAG-EF1a-CopGFP-Puro of the *TMED8* gene stored in a -20°C refrigerator was dissolved with 40 μL of DEPC water. An electric thermostatic water bath was heated to 42°C, while DH5α competent cells stored in a -80°C ultra-low temperature refrigerator were thawed on ice; when the competent cells were completely thawed, the transformation experiment was started immediately.

[0083] The overexpression plasmid of the *TMED8* gene (FIG. 16) was mixed with the thawed competent cells at a volume ratio of 1:3, mixed well by flicking the tube wall and blowing gently 2 to 3 times; a resulting mixture was allowed to stand on ice for 25 min, incubated in a 42°C water bath for exactly 45 s, and immediately transferred to ice for 3 min; a resulting transformed plasmid was transferred to a shaking tube containing 40 μL of antibiotic-free LB liquid medium to allow pre-shaking in a constant-temperature shaker at 37°C and 200 rpm for 1 h; 4 mL of a liquid medium containing ampicillin at a concentration of 50 μg/mL was added, and the bacterial cells were shaken at 250 rpm and 37°C overnight for 14 h to 16 h to avoid the growth of miscellaneous bacteria; a resulting bacterial solution was extracted using the Axygen plasmid extraction kit according to a standard procedure, and an obtained plasmid concentration was measured and recorded; the remaining bacterial solution could be mixed with glycerol to allow preservation and then stored at -80°C.

[0084] (2) Establishment of stably-transfected cell lines: an appropriate amount of wild-type cells to be transfected (PANC-1 and BXPC-3 pancreatic cancer cell lines) were inoculated in a 6-well plate, until a cell density reached 60% to 70% after 6 h of adherence; 80 μL of 1640 basal medium without serum and double antibodies were fused with 3 μg of the plasmid, mixed well by flicking the tube wall without pipetting with a pipette to avoid DNA breakage, and allowed to stand at room temperature for 5 min; 3μL of Lipofectamine 2000 transfection reagent was added, allowed to stand at room temperature for 20 min to 30 min, added into a complete medium of the 6-well plate, mixed well by gently pipetting with a pipette, and incubated in a cell incubator at 37°C and 5% carbon dioxide for 24 h to 48 h; the cell state was observed once in the first 24 h, and then observed once every 12 h, where once the cell status deteriorated due to the toxicity of the transfection reagent, the medium was changed immediately; after the transfection was stable, a cell transfection efficiency was observed using fluorescent tag of the plasmid under a fluorescence microscope; after the transfection was successful, the wild-type cells that had not been successfully transfected were screened with corresponding resistance, usually puromycin, and the successfully-transfected cells

were selected to establish a stably transfected cell line; after passage and preservation, a total cell protein or total RNA was collected for WB or qRT-PCR to verify the overexpression efficiency of the cells. The results were shown in FIG. 4.

[0085] 2. CCK-8 cell proliferation activity detection

[0086] The over-expressed stably transduced cells and wild-type control cell lines to be tested were separately pre-cultured in a medium dish, when the confluence of the cells in the dish reached 90%, the cells were digested and resuspended. It was noted that the amount of cells in the dish must be properly controlled since cells growing in a crowded state might trigger their own apoptosis, affecting the accuracy of subsequent results. The cells were counted and inoculated in a 96-well plate at 5,000 to 10,000 cells per well according to the cell growth characteristics. Each group was set with 5 to 6 replicate wells to reduce experimental errors caused by operating techniques. It was noted that the number of cells in the control group and the treatment group must be equal to achieve controlling variables. Detection of cell proliferation activity began 6 h to 12 h after the cells adhered to the wall.

Since cell proliferation activity needed to be continuously tested for 5 d based on the cell doubling cycle, experimental operations and result testing should be conducted at a same time point every day.

[0087] A CCK8 reagent was mixed with a basal medium of the corresponding cells at a volume ratio of 1:9 to obtain a fresh working solution. The medium in the original well was discarded. After washing with 1×PBS buffer, 100 μL of 10% CCK8 working solution was added to each well and allowed to stand for 1 h to 2 h in a 37°C incubator, and an absorbance at 460 nm was measured using a microplate reader. The data were recorded, and data processing and comparative analysis were conducted after all tests were completed.

[0088] The results were shown in FIG. 6. Overexpression of the *TMED8* gene inhibited the proliferation of pancreatic cancer cells.

[0089] 3. Cell scratching healing experiment

[0090] A brand new sterile packaged six-well plate was placed upside down on a clean workbench, a nine-square grid coordinate line was drawn on the bottom of the well plate using a ruler and a marker, so as to facilitate positioning for photos taken under a microscope for later scratch experiments. Appropriate amounts of wild-type and overexpression cell lines were inoculated in a six-well plate, with 3 replicate wells in each of the control group and overexpression group. According to the growth cycle, the cells were cultured with fresh complete nutrient medium to 90% density, and cell scratching was conducted with an alcohol-sterilized ruler and a 1 mL sterile blue pipette tip. During the cell scratching, it was noted that verticality and uniform strength were required, the size of the scratches in each repeated hole should be controlled to be basically equal to

reduce the result error caused by the operation and to better control the experimental variables. 3 scratch lines were also drawn in each well to ensure the repeatability of experimental results. After cell scratching was completed, 1×PBS buffer was added to clean the floating cells and cell debris with gentle force to prevent cells from being washed away at the scratch boundary. The complete medium with low serum concentration was replaced to maintain cell growth while controlling the growth rate for subsequent observation. A time point as 0 h from the start of the experiment was recorded, and photos were taken every 24 h. The healing status of cell scratches were observed under a microscope and photos were taken, and the final results of the experiment were integrated and analyzed 72 h later.

[0091] The results were shown in FIG. 5 and FIG. 6. Overexpression of the *TMED8* gene inhibited the migration ability of pancreatic cancer cells.

[0092] 4. Cell colony formation experiment

[0093] The cells to be tested were digested into a single state using trypsin containing EDTA, the cell suspension was collected, and the cells were counted. According to the number of 500 cells per well, the cells in the control group and the overexpression group were inoculated in 6-well plates, each group had 3 replicate wells. 2 mL of freshly cultured cells were added to each well and placed in a cell culture incubator, cultured at 37°C and 5% carbon dioxide for 5 d to 7 d. Since the initial number of cells was low, there was no need to change the medium during colony formation. After 5 d to 7 d, the culture plate was taken out to observe the colony formation status under a microscope. After washing with 1×PBS, 1 mL of 4% paraformaldehyde fixative was added to each well to fixate for 2 h, washed 3 times with PBS, and then stained with crystal violet dye for 1 h. The working solution was discarded, the cells were rinsed with double-distilled water and dried, and then photos were taken to observe the difference in colony formation between the two groups of cells.

[0094] The results were shown in FIG. 7 and FIG. 8. Overexpression of *TMED8* gene inhibited the formation of pancreatic cancer cell colonies, that is, inhibited the proliferation ability of pancreatic cancer cells.

[0095] 5. Transwell cell migration experiment

[0096] The cells to be tested were digested, resuspended, counted, and set aside for later use. A 24-well cell culture plate and the matching chamber with an 8 μm transparent PET film were taken, the chamber was placed in the culture well of the 24-well plate, and 600 μL of complete nutrient medium containing 5% fetal bovine serum was added outside the chamber slot or into the culture hole. 30,000 cells were counted and aspirated, fully resuspended in 200 μL of basal nutrient medium without fetal bovine serum, transferred with a pipette to the chamber, and cultured for 24 h to 72 h according to the cell growth cycle to allow the cells to fully penetrate the biofilm, and the migration ability of the cells was observed. The chamber was taken out, the medium inside was

discarded, and the chamber was placed in a clean 24-well plate, soaked in 4% paraformaldehyde fixative for 2 h, rinsed gently with double-distilled water, and then stained with crystal violet staining solution for 1 h. The cells were washed with double-distilled water after staining. If the staining was excessively and affected the observation of the results, methanol was added to allow decolorization and rinsing to elute the crystal violet in the blank areas of the membrane. After the staining was completed, the chamber was placed upside down, dried at room temperature, observed with an ordinary optical microscope, and photos were taken at a suitable field of view.

[0097] The results were shown in FIG. 5 and FIG. 6. Overexpression of the *TMED8* gene inhibited the migration ability of pancreatic cancer cells.

[0098] 6. Flow cytometry

[0099] Flow cytometry of cell apoptosis: PE fluorescein-labeled Annexin V dye and 7-AAD dye were added to double-stain the cells to be tested to detect cell apoptosis. First, the cells were digested and collected with EDTA-free trypsin, washed 2 to 3 times with 1×PBS cell washing solution, and counted. Each sample was generally controlled at 10,000 to 50,000 cells, without fixation to prevent subsequent quenching of fluorescence. The collected clean cell pellet was resuspended in 100 μL buffer, 1 μL of Annexin V dye and 5 μL 7-AAD dye were added to each 100 μL of suspension, mixed gently and stored in the dark at room temperature to allow staining at room temperature, reacted for 5 min to 10 min, then added with 400 μL of buffer, mixed well, transferred to a flow cytometer professional test tube, and the on-machine detection was completed within 1 h. PE was orange-red fluorescence, with an excitation wavelength of Ex=488 nm and an emission wavelength of Em=578 nm, and FL2 channel was recommended. 7-AAD dye was red fluorescent with an excitation wavelength of Ex=546 nm and an emission wavelength of Em=647 nm, and FL3 channel was recommended. Fluorescence compensation and adjustment were required during subsequent data analysis.

[0100] The results were shown in FIG. 9. Overexpression of *TMED8* gene promoted apoptosis of pancreatic cancer cells.

[0101] 7. Subcutaneous tumor formation in nude mice

[0102] 4-week-old male BALB/c immunodeficient nude mice were selected for subcutaneous tumor formation experiments. Before the experiment, each nude mouse was weighed and recorded, and the PANC-1 control cells and overexpressed cells were digested with trypsin and counted. It was appropriate to inject approximately 2×10^7 cells of each type into each nude mouse and resuspend in sterile 1×PBS solution. It was noted that the cell suspension must be placed on ice before animal experiments to avoid cell damage and death. Equal amounts of *TMED8*-overexpressing PANC-1 cells and PANC-1 control cells were injected into the left and right armpits of the upper limb of a same nude mouse. After that, the condition of the mice needed

to be observed every day. After 4 to 6 weeks of tumor formation, the nude mice were sacrificed and dissected, tumors were removed, weighed and measured, and then fixated for subsequent immunohistochemistry experiments. It was noted that conducting control experiments on the same nude mouse facilitated the control of interfering factors caused by mouse heterogeneity, thus more intuitively determining the impact of *TMED8* gene on tumor growth *in vivo*.

[0103] The results were shown in FIG. 10 and FIG. 11. The *TMED8* gene inhibited the tumor-forming ability of pancreatic cancer cells under the skin of nude mice, that is, inhibited the proliferation of pancreatic cancer cells in the animal body.

[0104] 8. Western Blot

[0105] (1) Extraction of total protein from cells and tissues: during the entire process, the cell samples to collect total protein should be placed on ice, the medium in the dish was discarded, and the cells were washed with 1×PBS solution, and added with 0.2 mL to 0.4 mL of RIPA effective lysis solution. After about half an hour of lysis, the cells were scraped with a cell scraper, transferred to a 1.5 mL centrifuge tube, and placed in an electric rotator at 4°C for rotation overnight to fully lyse the protein. The cells were centrifuged at 7,500 rpm for 15 min, the supernatant was collected to discard the cell debris, 1/4 of the total volume of the supernatant sample buffer (Loading Buffer) was added, and boiled for at least 30 min to ensure that the protein was fully heated and denatured. After cooling to room temperature, the protein was aliquoted and stored in -20°C or -80°C refrigerator to avoid protein degradation due to repeated freezing and thawing.

[0106] (2) Preparation of SDS-PAGE gel: the gel plate was washed, dried, and clamped on a gel preparation rack, double-distilled water was added to check for leaks. After passing the leak check, the gel plate was dried vertically and the gels were prepared according to the component proportions of separation gels of different concentrations (Table 2). The prepared separation gel was injected smoothly and evenly into the pre-set gel plate, paying attention to avoid the generation of air bubbles. The gel was pressed with isopropyl alcohol and allowed to stand for about 40 min, shaken gently to confirm that the colloid was solidified, then isopropyl alcohol was discarded and the gel plate was rinsed gently with double-distilled water and air-dried. The prepared upper stacking gel was added and a comb was quickly inserted vertically (Table 3). This step especially should avoid the generation of air bubbles, otherwise the sample well deformation and sample leakage might easily occur.

[0107] Table 2 Preparation of 10 mL SDS-PAGE separation gel components of different concentrations (mL)

[0108]

Component	6%	8%	10%	12%	15%

Double-distilled water	4.0	3.3	4.0	2.0	1.0
30% Acr-Bis	2.0	2.7	5.0	4.0	5.0
1.5 M Tris, pH=8.8	4.8	3.8	5.7	3.8	3.8
10% SDS	0.1	0.1	0.15	0.1	0.1
AP	0.1	0.1	0.15	0.1	0.1
TEMED	0.008	0.004	0.006	0.004	0.06

[0109] Table 3 Proportion scheme for preparing 8 mL upper layer stacking gel

[0110]

Component	Volume (mL)
Double-distilled water	5.5
30% Acr-Bis	1.3
1 M Tris, pH=6.8	1.0
10% SDS	0.08
AP	0.08
TEMED	0.008

[0111] 9. TMED8 may regulate the biological functions of PAAD tumor cells through the MAPK pathway and affect the occurrence and development of pancreatic cancer

[0112] PANC-1 control and overexpression cell lines (each group had 3 replicate samples) were selected for transcriptome sequencing. Through data analysis, 248 differential genes were obtained, of which 103 were up-regulated and 145 were down-regulated. These genes were visualized using heat maps and volcano plots separately, and functional annotation and KEGG enrichment analysis were performed. The MAPK signaling pathway with significant correlation was selected for further research, and the expression levels of key molecules ERK, JNK, P38 and their phosphorylation were verified by WB.

[0113] The results are shown in FIG. 10 to FIG. 15. *TMED8* gene inhibited the activation of the MAPK signaling pathway.

[0114] Although the above example has described the present disclosure in detail, it is only a part of, not all of, the examples of the present disclosure. Other examples may also be obtained by persons based on the example without creative efforts, and all of these examples shall fall within the protection scope of the present disclosure.

WHAT IS CLAIMED IS:

1. A reagent for detecting an expression level of a transmembrane emp24 domain 8 (TMED8) gene for use in diagnosing a tumor.

2. The reagent for use according to claim 1, wherein detection of the expression level of the TMED8 comprises detecting an expression level of an mRNA or a protein of the *TMED8* gene.

3. The reagent for use according to claim 1 or 2, wherein the reagent for detecting the expression level of the *TMED8* gene is at least one selected from the group consisting of a immunohistochemistry reagent, a Western Blot reagent, and a real-time fluorescence quantitative PCR reagent.

4. The reagent for use according to claim 1, wherein the tumor comprises pancreatic cancer.

5. A kit for diagnosing a tumor, comprising at least one of the following reagents: a reagent for detecting an expression level of a TMED8 protein using immunohistochemistry, a reagent for detecting the expression level of the TMED8 protein using Western Blot, and a primer pair for detecting an expression level of a *TMED8* gene and/or an mRNA using real-time fluorescence quantitative PCR.

6. The kit according to claim 5, wherein the primer pair for detecting the expression level of the TMED8 gene using real-time fluorescence quantitative PCR has the nucleotide sequence of SEQ ID NO: 1 and SEQ ID NO:2.

7. The kit according to claim 5 or 6, wherein when the expression level of the TMED8 gene is detected using real-time fluorescence quantitative PCR, primers for 18S as an internal reference, and the primers has the sequence of SEQ ID SEQ ID NO: 3 and SEQ ID NO:4.

8. A reagent for increasing an expression level of a *TMED8* gene for use in treating a tumor.

9. The reagent for use according to claim 8, wherein the reagent for increasing the expression level of the TMED8 gene is at least one selected from the group consisting of an agonist of the *TMED8* gene, an analog of the *TMED8* gene, and an agent for overexpressing the *TMED8* gene.

10. The reagent for use according to claim 9, wherein the agent for overexpressing the TMED8 gene comprises an TMED8 overexpression plasmid pLV3-CMV-TMED8(human)-3×FLAG-EF1a-CopGFP-Puro with the nucleotide sequence of SEQ ID NO: 5.

11. A drug used for treating pancreatic cancer, comprising an agent for increasing an expression level of an endogenous *TMED8* gene or an exogenous *TMED8* gene.

TRANSLATION

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2024/114759

A. CLASSIFICATION OF SUBJECT MATTER

C12Q1/6886(2018.01)i; G01N33/574(2006.01)i; A61K45/00(2006.01)i; A61P35/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC:C12Q,G01N,A61K,A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CJFD, CNTXT, ENTXT, ENTXTC, STN, PUBMED, SpringerLink, ISI Web of Knowledge, Elsevier Science Direct, Wiley InterScience, Nature, Science, CNKI, NCBI, 万方, WANFANG: SEQ ID NOs: 1-5, TMED8, pancreatic, cancer, diagnosing tumor, PC, 胰腺癌, 肿瘤, 诊断, MAPK信号通路

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	CN 116769916 A (AFFILIATED HOSPITAL OF NANTONG UNIVERSITY) 19 September 2023 (2023-09-19) claims 1-10	1-14
PX	WO 2023162878 A1 (NIPPON MEDICAL SCHOOL FOUNDATION) 31 August 2023 (2023-08-31) claims 1-15	1-14
X	CN 101541977 A (NOVARTIS AG) 23 September 2009 (2009-09-23) claims 1-7, and description, pages 16-18 and 497-500, and table VIII	1-14
X	US 2014342946 A1 (MONI ABRAHAM KURIAKOSE et al.) 20 November 2014 (2014-11-20) claims 1-7, and description, paragraphs 7-18, and table v	1-7
A	CN 115678995 A (SHANGHAI IPROTEOME BIOLOGICAL TECHNOLOGY CO., LTD.) 03 February 2023 (2023-02-03) claims 1-8	1-14

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 November 2024	**20 November 2024**
Name and mailing address of the ISA/CN **China National Intellectual Property Administration (ISA/CN)** **China No. 6, Xitucheng Road, Jimenqiao, Haidian District, Beijing 100088**	Authorized officer Telephone No.

TRANSLATION

INTERNATIONAL SEARCH REPORT

International application No.
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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 116004810 A (SHENZHEN LUWEI BIOTECHNOLOGY CO., LTD.) 25 April 2023 (2023-04-25) claims 1-7	1-14
A	GUO, X.L. et al. "TMED3 Promotes the Development of Malignant Melanoma by Targeting CDCA8 and Regulating PI3K/Akt Pathway" *Cell & Bioscience*, Vol. vol. 13, 29 March 2023 (2023-03-29), Article 65, abstract	1-14

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International application No.
PCT/CN2024/114759

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed.
   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

TRANSLATION

INTERNATIONAL SEARCH REPORT

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **12-14**
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 12-14 relate to a method for treating tumors, which falls within methods for treatment of diseases as defined in PCT Rule 39.1(iv). However, a search is still performed on the basis of the pharmaceutical use thereof.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

TRANSLATION

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2024/114759

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	116769916	A	19 September 2023	None			
WO	2023162878	A1	31 August 2023	JPWO	2023162878	A1	31 August 2023
CN	101541977	A	23 September 2009	JP	2010504530	A	12 February 2010
				AU	2007340265	A1	10 July 2008
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				KR	20090071603	A	01 July 2009
				MX	2009002936	A	01 April 2009
				BRPI	0716944	A2	17 September 2013
				IL	197450	A0	24 December 2009
				US	2010004253	A1	07 January 2010
				RU	2009114745	A	27 October 2010
				CA	2662508	A1	10 July 2008
				JP	2013128487	A	04 July 2013
				WO	2008082730	A2	10 July 2008
				WO	2008082730	A3	08 January 2009
				EP	2074226	A2	01 July 2009
				SG	174826	A1	28 October 2011
US	2014342946	A1	20 November 2014	IN	4915/CHENP2014	A	18 September 2015
				WO	2013098797	A2	04 July 2013
				WO	2013098797	A3	12 September 2013
CN	115678995	A	03 February 2023	None			
CN	116004810	A	25 April 2023	None			