Transcriptomic Analyses and Potential Therapeutic Targets of Pancreatic Cancer With Concomitant Diabetes

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Background: Type 2 diabetes mellitus (T2DM) known as non-insulin-dependent diabetes mellitus, which is increasingly acknowledged as being associated with an increased risk for a series of cancers. Pancreatic cancer is currently the fourth most common cause of cancer-related mortality, which has been proved to be worsened by internal diabetic condition. However, the underlying molecular mechanisms are less addressed. Furthermore, current knowledge revealed that therapeutic strategy by anti-diabetes for pancreatic cancer under diabetes condition have no satisfactory efficacy, and nor by chemotherapy in our study.

Methods: To clarify these mysteries and widen our knowledge, both obesity-associated and non-obese-associated T2DM mouse models were generated by chemical induction with streptozotocin (STZ) and leptin receptor knockout (db/db) in mice. Then, the process of tumor progression was researched, and the gene expression profiling of pancreatic cancer in mice was performed using RNA-seq.

Results: Our results showed that pancreatic cancer malignancy was increased with notable proliferation and metastatic potential in two diabetic mice model. Totally, 136 and 64 significantly differentially expressed genes (DEGs) were identified in STZ and db/db mice by transcriptomic analysis. The results also suggested that different carcinogenesis-related genes and potential molecular mechanisms contribute to the malignancy of pancreatic cancer in obesity-associated and non-obesity-associated T2DM. In obesity-associated db/db mice, the GO subcategories associated with most of the genes with downregulated expression are involved in the immune response. However, in non-obesity-associated STZ mice, in addition to the immune response category, the enriched subcategories also included angiogenesis and the extracellular matrix. While, two genes respectively encoding MMP-2 and MMP-9 were simultaneously abnormal upregulated in pancreatic cancer tissue from diabetic mice of both STZ and db/db, that could act as potential therapeutic targets for significantly suppressing the malignant progression. Furthermore, an optimizing therapeutic strategy was further proposed that
INTRODUCTION

Type 2 diabetes mellitus (T2DM) is known as non-insulin-dependent diabetes mellitus or adult-onset diabetes and is the most common form of diabetes, affecting approximately 90% to 95% of all patients diagnosed with diabetes (1). With the rapid increase in the incidence of diabetes worldwide, the prevalence of diabetes in combination with cancer is increasingly being observed (2, 3). Convincing evidence indicates that diabetes, particularly T2DM, is associated with an increased risk of the development and progression of several cancers, such as breast, liver, pancreas, colorectal, and bladder cancer (4, 5). A possible explanation for the link between cancer and diabetes is that these two disorders share many potential modifiable (e.g., weight change, diet, physical activity, tobacco smoking, and alcohol) and non-modifiable (e.g., sex, age, and race) risk factors (6, 7). As research has progressed, an increasing number of studies have focused on the possible biological links between diabetes and cancer, including several mechanisms such as hyperinsulinaemia (either endogenous or exogenous), hyperglycaemia, and chronic inflammation (8–11). In addition, another critical issue regarding the association between T2DM and cancer is whether drug use in patients with T2DM is causally associated with cancer. Clinically, medication-based diabetes treatments have been used, and a reduced risk of developing cancer has been reported in patients treated with several anti-diabetic drugs, such as metformin (12). However, anti-diabetes strategies may also not prevent or may even promote cancer development. For example, clinical trial evidence has shown that an increased malignancy rate is associated with the long-term use of exogenous insulin (13), and the postulated links between incretin-based therapies, such as glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors, and pancreatic cancer have not been substantiated (14).

Pancreatic cancer is currently the fourth most common cause of cancer-related mortality (2, 15), and predictions indicate that it will be the second leading cause of cancer-related mortality within the next decade (3). Most patients are diagnosed at an advanced stage with distant metastasis and/or locally advanced unresectable tumors, and the 5-year survival rate is less than 7% (16). Diabetes mellitus has been identified as a risk factor for pancreatic cancer, causing increased morbidity and mortality and manifesting as an increased tumor size and reduced survival (17–20). In addition, some research has suggested that diabetes is both a cause and a consequence of pancreatic cancer (21), as pancreatic cancer itself induces diabetes (type 3c). The potential mechanisms of this process include the release of adrenomedullin, a potential mediator of beta cell dysfunction (22) and beta cell apoptosis induced by pancreatic stellate cells (23). Currently, anti-diabetes therapeutic strategies for pancreatic cancer patients with diabetes are not effective (4, 24–26), and there is a lack of effective therapeutic strategies.

Carcinogenesis is a complex process that involves multiple steps, including initiation, promotion and progression. A number of genes and proteins, for example, KRAS, CDKN2A, TP53, HER2, and HIF-1α, have been indicated to play important roles in those steps (27–30). One or more genes and proteins might be more abnormally expressed in pancreatic cancer with concomitant diabetes, resulting in the promotion of cancer progression. Thus, in this study, we attempted to identify potential genes and proteins that were abnormally expressed and then to target/suppress these genes and proteins to search for a more effective therapeutic strategy. To search for abnormally expressed genes, transcriptomic analyses were performed. In addition, being overweight or obese is considered the principal modifiable risk factor for diabetes (31); in 2010, 84.7% of adults aged ≥18 years with diagnosed diabetes in the United States were overweight or obese (32). Diabetes is an increasingly prevalent chronic condition in Asia despite low obesity rates and low body mass indexes (BMIs) (33, 34). Thus, to completely account for the various types of T2DM in humans, obese and nonobese mouse models of diabetes were established and studied.

MATERIALS AND METHODS

Animal Models of Diabetic Pancreatic Cancer

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Medical School of Southeast University. All mice were purchased from Kawensi Limited Company in Changzhou, China. Wild-type C57BL/6J mice (male, 8–10 weeks old) were used as nondiabetic controls (WT). Diabetic mouse models were generated either by chemical induction with streptozotocin (STZ) or through systemic mutations of leptin receptor (db/db). Since STZ-induced diabetes is not considered to be completely representative of T2DM in humans, especially obesity-associated T2DM, db/db...
We used the db/db mouse as the model of diabetes to simulate the pathophysiology of obesity- and non-obesity-associated T2DM, respectively. Mice with the db/db genetic background develop hyperglycaemia as a consequence of obesity due to the deficiency in leptin receptor and beta cell failure and naturally exhibit many of the symptoms observed in obesity-associated T2DM patients. The STZ mouse model of diabetes was constructed as described by Chow et al. (35). The weights of the animals in the different model groups were measured. The weights of db/db mice were significantly higher than those of WT mice, whereas those of STZ mice were significantly lower than those of WT mice.

To verify that T2DM was successfully induced in these animals, glucose levels in blood collected from the tail vein were measured using a glucometer (Roche Diagnostics GmbH, Mannheim, Germany). The results showed that the glucose levels in WT mice were 6.88 ± 0.57 mmol/L (n=6), which were significantly lower than those in diabetic mice from the STZ (n=7, 23.67 ± 2.92 mmol/L; t=-5.212, df=11, p<0.001) and db/db (n=10, 20.65 ± 2.12 mmol/L; t=-4.893, df=14, p<0.001) groups.

Orthotopic pancreatic cancer models were generated in WT, STZ and db/db mice as previously described (36). Briefly, murine pancreatic cancer panc02 cells purchased from the Cell Bank of Shanghai Institute of Biological Science (Shanghai, China) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) with 10% foetal bovine serum (FBS) and 1% penicillin and streptomycin. To monitor tumor development, migration, and therapeutic response in vivo, optical imaging reporter molecules were introduced into Panc02 cells with effective lentiviral infection procedures that were previously used in our laboratory (37). Briefly, with the FUGW-Luc2-eGFP construct generated using an hUBC-driven eGFP lentiviral plasmid and an FUGW vector, Panc02 cells were labelled with green fluorescent protein (GFP) and firefly luciferase dual reporters. Then, GFP-positive cells were sorted using a FACSChirub instrument (BD Biosciences, USA). Panc02 cells were harvested and resuspended as single-cell suspensions in 100 µl and then were slowly injected into the distal femurs of WT mice. When subcutaneous tumors reached 8–10 mm in diameter, 1-mm3 tissue fragments were surgically resected from the subcutaneous tumor graft and were implanted into the pancreatic tail of WT STZ and db/db mice to obtain orthotopic pancreatic cancer models.

**Pancreatic Cancer Progression in Diabetic Mouse Models**

Tumor growth was monitored via magnetic resonance imaging (MRI) using a 7.0 Tesla MR scanner (Bruker Pharma Scan; Ettlingen, Germany) with a transmit-receive quadrature volume coil. T2-weighted imaging (T2WI) using a fast spin-echo sequence with respiratory gating (echo time 36 ms) was performed on mice for 12 days after tumor implantation. The acquisition parameters included a field of view of 32×32 mm, a matrix of 256×256, and a total of 24 slices with a 1-mm slice thickness. Tumor length and width were manually measured in the largest cross-sectional area of the T2-weighted images to calculate the tumor volume according to the formula V=(width²×length)/2 (38).

At the end of the MRI experiment, mice were anaesthetized for in vivo bioluminescence imaging. Before imaging, D-luciferin (intraperitoneal (i.p.), 150 mg/kg; Promega, USA) was administered and allowed to react with the substrate for 10 min. An IVIS Spectrum imaging system (PerkinElmer, USA) was used to acquire bioluminescence images. Abdominal metastases were counted in the bioluminescence images.

**Therapeutic Schedule for Gemcitabine**

A standard treatment for pancreatic cancer, gemcitabine (Sigma, USA), which is the first-choice chemotherapy agent for pancreatic cancer (39), was used to treat WT (n=10), STZ (n=7), and db/db (n=8) mice. Mice were administered gemcitabine (25 mg/kg, i.p.) every 3 days for 20 days. The tumor volume and metastases were evaluated using the methods described above. The control groups mice were administered an equal volume of vehicle (10% DMSO).

**Transcriptomic Analyses**

For the transcriptomic experiment, tumor tissue (3 mm³) that was transplanted in WT, STZ and db/db mice for 12 days was collected and pooled in a plastic tube (1.5 ml), snap-frozen in liquid nitrogen, and transferred to a −80°C freezer for long-term storage. RNA from each sample group was extracted with TRIzol reagent (Invitrogen; USA), and each group included three replicates. The quality of the isolated RNA was assessed using a NanoDrop (Thermo Scientific NanoDrop 2000, USA), and the A260/280 values were all above 2.0. A total of 3 μg of total RNA from each sample was converted to cDNA using an NEBNext® Ultra™ RNA Library Prep kit for Illumina® (NEB, USA). In total, nine cDNA libraries were constructed and subsequently sequenced with an Illumina HiSeq 2000 platform by Beijing Biomarker Technologies Co., Ltd., resulting in raw reads. Clean reads were obtained by removing reads containing adapters, poly-N reads and low-quality reads from the raw data using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/), and these clean reads were used for further analyses. Approximately 44.24 million clean reads were obtained from each sample. The Q30 was greater than 93.57% in each sample, showing that the sequences of each sample were of high quality.

The transcriptome was assembled using StringTie (40) based on the reads that mapped to the reference genome GRCh38; more than 93.18% of the reads were successfully mapped. In addition, more than 2,947 new genes were found. For functional annotation, BLASTX was used to compare the new genes to eight public databases. A total of 1,867 new genes were successfully annotated, which included 223 genes in Clusters of Orthologous Genes of proteins (COG), 843 genes in Gene Ontology (GO), 330 genes in Kyoto Encyclopedia of Genes and Genomes (KEGG), 439 genes in the EuKaryotic Orthologous Groups (KOG), 655 genes in Pfam, 879 genes in SWISS-PROT, 1232 genes in EggNOG, and 1854 genes in NR.

We putatively identified expressed genes with RSEM using the reads per kb per million reads (RPKM) method (41). Genes with at least a 2-fold expression change (i.e., log2fold change (FC)≥2) and a false discovery rate (FDR)<0.01, as found by the DESeq R package (1.10.1), were considered differentially expressed. The GOseq R package and KOBAS software were
used to implement the statistical enrichment of differentially expressed genes (DEGs) in the GO and KEGG pathways, respectively, and an adjusted Q-value < 0.05 was chosen as the significance cutoff (42).

**Expression of MMP-2 and MMP-9 in Tumors From Mice**

To investigate MMP-2 and MMP-9 expression, Western blotting and immunohistochemistry (IHC) were performed on tumor tissue samples collected 12 days after tumor transplantation. Protein expression was analysed using antibodies against MMP-2 (ab37150, 1:500; Abcam, UK), MMP-9 (ab38898, 1:1000; Abcam, UK), and β-actin (1:2000; Abcam, UK). IHC was performed according to the manufacturer’s recommended protocol using rabbit anti-mouse MMP-9 (ab38898; Abcam, UK) and MMP-2 (ab37150; Abcam, UK) antibodies, and the integrated optical density (OD) intensity of IHC staining was quantified as previously reported (43).

**Therapeutic Schedules for a Selective MMP-2/9 Inhibitor**

In addition, WT (n=7), STZ (n=9) and db/db (n=9) mice with pancreatic cancer were given an i.p. injection of the selective MMP-2/9 inhibitor SB-3CT (Selleck, USA) diluted in 10% DMSO at a dosage of 25 mg/kg daily for 20 days (44). The control groups for WT (n=9), STZ (n=9) and db/db (n=11) mice were administered an equal volume of vehicle (10% DMSO). Tumor growth was monitored using T2-weighted MRI. Metastatic lesions were counted in bioluminescence images. Western blotting was conducted to evaluate MMP-2 and MMP-9 expression levels.

**Therapeutic Schedules for the Combination Therapy**

To investigate the potential synergistic effect of the MMP-2/9 inhibitor SB-3CT and gemcitabine combination therapy, WT (n=9), STZ (n=8), and db/db (n=8) mice were administered SB-3CT (25 mg/kg, i.p.) daily for 20 days. During this period, gemcitabine (25 mg/kg, i.p.) was injected every 3 days. Tumor volume and metastases were evaluated using the methods described above.

**Statistical Analysis**

One-way ANOVA followed by Tukey’s test was used to examine the differences in tumor volume, the OD intensity of IHC staining (MMP-2 and MMP-9), tumor-background ratio (TBR) values, and the number of metastatic nodules in animals. A value of $p \leq 0.05$ was considered statistically significant. All analyses were performed with SPSS v.20 software (IBM SPSS, Armonk, NY, USA).

**RESULTS**

**Pancreatic Cancer Progression in Diabetic Mouse Models**

The process of tumor progression was first investigated. Similar to previous reports, the pancreatic cancer volume in STZ (n=12, 257.70 ± 32.28 mm$^3$; $t=-3.027$, $df=24$, $p<0.05$) and db/db (n=14, 312.33 ± 41.01 mm$^3$; $t=-3.593$, $df=26$, $p=0.001$) mice was significantly larger than that in WT mice (n=14, 156.89 ± 13.77 mm$^3$) (Figure 1A).

In addition, we found that the number of metastases in STZ (n=9, 32.00 ± 5.83; $t=3.797$, $df=15$, $p<0.05$) and db/db (n=10, 21.80 ± 7.38; $t=-1.625$, $df=16$, $p<0.05$) mice was significantly higher than that in WT mice (n=8, 8.25 ± 0.86) (Figures 1B, C).

**Gemcitabine Treatment**

Standard chemotherapy, i.e., gemcitabine, is the first-choice chemotherapy agent for pancreatic cancer (39), which was used as a treatment in our study. The results showed that the tumor volume significantly decreased in WT, STZ and db/db mice with gemcitabine treatment (all $p<0.05$). However, gemcitabine was less effective at reducing the number of metastases (Figure 2).

**Transcriptome Analyses**

For enhancing our knowledge, the gene expression profiling of pancreatic cancer under diabetes, was performed using RNA-seq. When comparing pancreatic cancer tissue from non-diabetic WT mice with that from obesity-associated diabetic db/db mice, a total of 64 significant differentially expressed genes (DEGs) were identified (Table S1). The expression of 21 and 43 genes was up- and downregulated, respectively, in pancreatic cancer tissue from db/db mice (Figure 2). In addition, a total of 136 DEGs were identified when comparing pancreatic cancer tissue from WT mice with that from non-obesity-associated STZ-induced diabetic mice, and the expression of 50 and 86 genes was up- and downregulated, respectively, in pancreatic cancer tissue from STZ mice (Table S2).

**GO Enrichment Analyses of DEGs in db/db Mice vs. WT Mice**

GO enrichment analyses generated two subcategories: interleukin (IL)-8 receptor activity (GO: 0004918; $p<0.05$) and enzyme binding (GO: 0019899; $p<0.05$), in which the genes with upregulated expression were enriched. Furthermore, nine subcategories were enriched in genes with downregulated expression; most of these downregulated gene-enriched subcategories, for example, MHC class II protein complex (GO: 0072558; $p<0.05$), interleukin-1α production (GO: 0032610; $p<0.01$), natural killer (NK) cell activation (GO: 0030101; $p<0.05$), NLRP1 inflammasome complex (GO: 0072558; $p<0.01$), and positive regulation of interleukin-1β secretion (GO: 0050718; $p<0.01$) are involved in the immune response (Table 1).

The immune response is an important element in the progression of pancreatic cancer (45). Tumor cells exploit several immunosuppressive mechanisms to block the antitumoral response (46–48). For example, in the tumor microenvironment, the cell-surface or secreted form of IL-1α is associated with anti-tumor responses (49), and NK cells are important cytotoxic cells in the immune system and can recognize and kill cancer cells (50). While, associated genes belonging to those GO enrich subcategories were downregulated in pancreatic cancer tissue from db/db mice. It was suggested that
FIGURE 1 | STZ-induced or genetically induced Type 2 diabetes mellitus (T2DM) promotes pancreatic cancer progression. (A) Tumor volume was visualized and quantified according to T2-weighted images, which were acquired by MR scans, on day 12 after tumor implantation from WT, STZ, and \( \text{db/db} \) mice. Representative T2-weighted images are presented. The red circle indicates the tumor location. (B) Pancreatic cancer metastasis in WT, STZ and \( \text{db/db} \) mice was evaluated with bioluminescence imaging on day 12 after tumor implantation. (C) Representative \textit{in vivo} and \textit{ex vivo} bioluminescence imaging are shown. Scale bar, 1 cm. Data are presented as the mean ± S.D.

FIGURE 2 | Antitumor effects in diabetic mice with pancreatic cancer after treatment with gemcitabine (Gem). (A, B) Gem (25 mg/kg, Sigma, i.p.) was administered every 3 days for 20 days in WT, STZ, and \( \text{db/db} \) mice with pancreatic cancer after tumor transplantation. Tumor volume was quantified in non-diabetic and diabetic mice with pancreatic cancer based on T2-weighted images. Representative tumor volumes based on T2-weighted images are shown from each group (A), and tumor volumes at 20 days after Gem treatment were calculated in each group (B). The red circle indicates the tumor location. (C, D) Bioluminescence was analyzed in each group after Gem treatment to visualize and evaluate tumor metastasis, and the tumor metastasis number from each group at 20 days after Gem treatment was calculated (C). Representative \textit{in vivo} bioluminescence imaging is shown (D). The control groups mice were administered an equal volume of vehicle (Veh). Data are shown as the mean ± S.D.
the attenuated immune response in db/db mice with obesity-associated T2DM might be an important biological factor leading to the increased malignancy of pancreatic cancer.

**GO Enrichment Analyses of DEGs in STZ Mice vs. WT Mice**

GO enrichment analyses generated 16 enriched subcategories of genes with downregulated expression and 10 enriched subcategories of genes with upregulated expression, and are listed in detail in Table 2. Many of the enriched subcategories are involved in carcinogenesis, could be mainly divided into 3 parts, and are discussed as follows:

In addition to the enriched subcategories (e.g., MHC class Ib protein complex, GO: 0002685; cellular response to interferon-gamma, GO: 0071346) involved in the immune response associated with downregulated genes, enriched subcategories of "collagen type I trimer" (GO: 0005584) and "extracellular matrix structural constituent" (GO: 0005201) associated with the ECM were identified. A number of studies have shown that changes in the levels of ECM proteins play a major role in invasion and migration and correlate with the increased invasive potential of pancreatic cancer cells (51–54).

In addition, subcategories (e.g., vascular endothelial growth factor receptor binding, GO: 0005172; regulation of endothelial cell proliferation, GO: 0001936; and glomerulus vasculature development, GO: 0072012) involved in tumor vasculature and angiogenesis were enriched in genes with upregulated expression. Activation of the vascular endothelial growth factor (VEGF)/VEGF-RII pathway has been shown to regulate angiogenesis, and tumor vasculature and angiogenesis play an important role in tumor growth, metastasis and chemotherapeutic and radiotherapy responses (55, 56). Therefore, the abovementioned

| TABLE 1 | GO enrichment analyses in db/db vs. WT mice. |
| --- | --- | --- | --- |
| Ontology | GO_ID | GO_term | p |
| Downregulated genes | Molecular Function | GO:0019904 | protein domain specific binding | 0.015145 |
| Cellular Component | GO:0072558 | NLRP1 inflammasome complex | 4.51E-06 |
| GO:0042613 | MHC class II protein complex | 0.018398 |
| Biological Process | GO:0032810 | interleukin-1 alpha production | 8.24E-07 |
| GO:0070266 | Pyroptosis | 0.000135 |
| GO:0032406 | response to muramyl dipeptide | 0.000037 |
| GO:0050718 | positive regulation of interleukin-1 beta secretion | 0.01845 |
| GO:0030163 | protein catalytic process | 0.004336 |
| GO:0030101 | natural killer cell activation | 0.038056 |
| Upregulated genes | Molecular Function | GO:0004918 | interleukin-8 receptor activity | 0.040448 |
| GO:0019899 | enzyme binding | 0.047501 |

| TABLE 2 | GO enrichment analyses in STZ vs. WT mice. |
| --- | --- | --- | --- |
| Ontology | GO_ID | GO_term | p |
| Downregulated genes | Molecular Function | GO:0004055 | argininosuccinate synthase activity | 0.002019 |
| GO:0005201 | extracellular matrix structural constituent | 0.042346 |
| GO:0042606 | peptide antigen binding | 0.046186 |
| Cellular Component | GO:0009584 | collagen type I trimer | 0.001452 |
| GO:0002398 | MHC class Ib protein complex | 0.021541 |
| GO:0005615 | extracellular space | 0.026812 |
| GO:0070852 | cell body fiber | 0.039994 |
| Biological Process | GO:0071366 | cellular response to tumor necrosis factor | 0.000209 |
| GO:0071346 | cellular response to interferon-gamma | 0.000209 |
| GO:0042832 | defense response to protozoan | 0.000135 |
| GO:0006526 | arginine biosynthetic process | 0.019691 |
| GO:0000053 | argininosuccinate metabolic process | 0.019691 |
| GO:0071230 | cellular response to amino acid stimulus | 0.035324 |
| GO:0007494 | midgut development | 0.039285 |
| GO:0020092 | negative regulation of natural killer cell chemotaxis | 0.039285 |
| GO:0084016 | response to growth hormone | 0.046176 |
| Upregulated genes | Molecular Function | GO:0005172 | vascular endothelial growth factor receptor binding | 0.019203 |
| Biological Process | GO:0014842 | regulation of satellite cell proliferation | 0.002814 |
| GO:0030210 | heparin biosynthetic process | 0.009355 |
| GO:0033552 | response to vitamin B3 | 0.014015 |
| GO:0007171 | activation of transmembrane receptor protein tyrosine kinase activity | 0.026095 |
| GO:0003160 | endocardium morphogenesis | 0.026095 |
| GO:0001836 | regulation of endothelial cell proliferation | 0.033509 |
| GO:0072012 | glomerulus vasculature development | 0.033509 |
| GO:0031398 | positive regulation of protein ubiquitination | 0.041058 |
| GO:0043014 | Tie signaling pathway | 0.041333 |
biological factors predominantly involved in the immune response, angiogenesis and ECM remodeling are likely to commonly contribute to the malignancy of pancreatic cancer in non-obese-associated T2DM, i.e., STZ-treated mice.

**KEGG Pathway Enrichment Analyses**

Consistent with the results of GO enrichment in db/db mice, pathway enrichment tests revealed that immune response-related pathway, NOD-like receptor signaling pathway (ko: 04621, Table S3) was enriched in genes with downregulated expression. While, in STZ mice, enriched functional categories, e.g., ECM-receptor interaction (ko: 04512), platelet activation (ko: 04611), Rap1 signaling pathway (ko: 04015), adherens junction (ko: 04520) (Table S3), mainly involved in carcinogenesis, which might contribute to the malignancy of pancreatic cancer in STZ-treated mice. In general, results of KEGG pathway enrichment are roughly consistent with the results of GO enrichment analyses.

**DEGs Related to Carcinogenesis**

Consistent with previous reports (23, 57, 58), in our study, pancreatic cancer malignancy (i.e., measured by tumor volume and metastasis number) was found in both obesity-associated db/db T2DM mice and non-obese-associated STZ T2DM mice. It is generally hypothesized that diabetes may influence this neoplastic process by several mechanisms associated with hyperinsulinaemia, hyperglycaemia, and chronic inflammation (8–11). Carcinogenesis is a complex process, which involves multiple steps, including initiation, promotion and progression and is mediated by a series of genes (e.g., KRAS, CDKN2A, TP53, HER2, and HIF-1α) (27–30). Our transcriptomic data identified many genes i.e., DEGs that have been reported in previous studies to be associated with carcinogenesis (Tables S1 and S2), which were significantly differentially expressed in the pancreatic cancer samples from STZ or db/db mice, or both. These DEGs could be candidate genes associated with the increased malignancy of pancreatic cancer in T2DM and may serve as potential therapeutic targets, as well. Further work should be conducted to identify genes playing a critical role and to identify effective therapeutic targets from these genes.

Among the DEGs, 11 were significantly differentially expressed in pancreatic cancer samples from both STZ and db/db mice, with the same expression tendencies (Table 3). Consistent with the results of the GO enrichment analysis, most genes associated with carcinogenesis in STZ and db/db showed many expression differences; for example, Cdc42 gene was upregulated in STZ mice but showed no expression differences in db/db mice (Tables S1 and S2). This finding suggests that although the pancreatic cancer malignancy was increased in both obese-associated db/db T2DM and STZ mice with non-obese-associated T2DM (Figure 1), the increase may be caused by different genes and may also involve different mechanisms. In addition, precise and differentiated therapeutic targets may be selected according to whether pancreatic cancer patients have obesity- or non-obesity-associated T2DM.

In addition to selecting differentially expressed candidate genes for therapeutic targets, we also attempted to explore the common targets according to the 11 genes differentially expressed in pancreatic cancer from both db/db and STZ mice for both obese- and non-obese-associated T2DM. For example, the expression of MMP-2 and MMP-9 in the abovementioned 11 DEGs was found to be upregulated in the pancreatic cancer samples from both STZ and db/db mice and were further researched in subsequent analyses (see Therapeutic Targets of MMP-2 and MMP-9). Theoretically, common therapeutic targets have a wide range of applications for pancreatic cancer in both non-obese- and obese-associated db/db T2DM patients.

**Expression of MMP-2 and MMP-9 in Tumors From Mice**

Transcriptomic data further showed, as previously described (results of qPCR) (58), that the expression of the genes encoding MMP-2 and MMP-9 was upregulated in pancreatic cancer tissues from both STZ and db/db mice; this result was also confirmed by Western blotting (Figures 3A, B) and IHC analyses (Figures 3C, D, all p<0.05).

**Therapeutic Targets of MMP-2 and MMP-9**

When STZ and db/db mice bearing pancreatic cancer were treated with a selective inhibitor of MMP-2 and MMP-9, SB-3CT, for 20 days in this study, the expressions of MMP-2 and MMP-9 significantly decreased (all p<0.05) (Figures 4A–C). In addition to tumor volume changes (58), the number of metastases significantly decreased, as well (Figures 4D, E).

| ID          | Gene name           | WT vs. STZ | WT vs. db/db | NR annotation                                                                 |
|-------------|---------------------|------------|--------------|--------------------------------------------------------------------------------|
| ENSMUSG000000061577 | Gpr114        | Down       | Down         | probable G-protein coupled receptor 114 isoform 1 precursor [Mus musculus]     |
| ENSMUSG000000021200 | Asb2          | Down       | Down         | ankyrin repeat and SOCS box protein 2 [Mus musculus]                          |
| ENSMUSG000000015852 | Fcris          | Up         | Up           | Fc receptor-like S, scavenger receptor precursor [Mus musculus]                |
| ENSMUSG000000017737 | Mmp9          | Up         | Up           | matrix metalloproteinase-9 precursor [Mus musculus]                           |
| ENSMUSG000000058126 | Tpms3-rs7     | Down       | Down         | trompomysin alpha-3 chain isoform 3 [Mus musculus]                            |
| ENSMUSG000000091679 | Vmn2r96       | Down       | Down         | vomeronasal 2, receptor 96 [Mus musculus]                                     |
| ENSMUSG000000011740 | Mmp2          | Up         | Up           | 72 kDa type IV collagenase precursor [Mus musculus]                           |
| ENSMUSG000000022501 | Pim1          | Down       | Down         | sperm proteamine P1 [Mus musculus]                                            |
| ENSMUSG000000055546 | Tmtd4         | Down       | Down         | T-cell immunoglobulin and mucin domain-containing protein 4 precursor [Mus musculus] |
| ENSMUSG000000023827 | Agmat4        | Up         | Up           | 1-acetyl-sn-glycerol-3-phosphate acyltransferase delta [Mus musculus]         |
| ENSMUSG000000036007 | Acan          | Up         | Up           | aggrecan core protein precursor [Mus musculus]                               |
Comparison of the Therapeutic Effect of an MMP-2/9 Inhibitor and Gemcitabine

Additionally, to better understand and compare the therapeutic effect of targeting MMP-2 and MMP-9 with SB-3CT, a standard chemotherapy for pancreatic cancer, i.e., gemcitabine, was used. The results showed that compared with inhibitor treatment, gemcitabine treatment had a greater effect on tumor volume (Figures 5A, B); however, gemcitabine was less effective at reducing the number of metastases (Figures 5C, D).

Optimizing the Therapeutic Strategy by Combining an MMP-2/9 Inhibitor With Gemcitabine

Unlike primary tumors, metastasis is largely incurable and contributes to more than 90% of cancer-associated tumors (59). Considering the abovementioned shortcomings of using chemotherapy or the inhibitor alone, a therapeutic strategy combining these two treatments was implemented. The results showed that the combination therapy was significantly more effective than either gemcitabine or MMP-2/9 inhibitor treatment alone for both non-obesity- and obesity-associated diabetic pancreatic cancer, resulting in the smallest tumor volume and lowest number of metastases (Figure 5, all p<0.05).

DISCUSSION

An increasing amount of evidence has shown that diabetes is an important risk factor for many cancers (2–5), including pancreatic cancer, as higher morbidity and lower survival has been observed in pancreatic cancer patients with T2DM (17–20). This study provides supporting evidence for the promotion of pancreatic cancer progression by diabetes, with accelerated tumor growth and a higher degree of metastasis in diabetic individuals. There are a number of shared risk factors in both diabetes and cancers, such as obesity and hyperinsulinaemia, that have been considered as possible explanations for this link (6–11). Our study also provides supporting evidence that obesity is a potential links between diabetes and pancreatic cancer, as in our...
study, obesity-associated \(db/db\) mice presented more aggressive pancreatic tumor progression than non-obesity-associated STZ mice.

Currently, a growing amount of observational evidence suggests that therapies for diabetes can decrease the incidence and mortality of cancer (e.g., breast cancer) in patients with diabetes (12). However, recent studies have shown that the risk of pancreatic cancer might not be suppressed by anti-diabetes therapy (14). In addition, the standard chemotherapy for pancreatic cancer, i.e., gemcitabine, was used as well, but the efficacy was not satisfactory. As shown in many clinical studies (22, 23), diabetic individuals who undergo adjuvant chemotherapy for pancreatic cancer present with larger tumors (also see our results) and have a smaller reduction in the metastasis number. Thus, the unsatisfactory efficacy of current therapeutic strategies inspired us to explore other treatment strategies or therapies.

Carcinogenesis is a complex process that involves multiple steps, including initiation, promotion and progression, and is mediated by a number of genes and proteins (27–30). Thus, when diabetes promoted the progression of cancer, transcriptomic analysis results showed that there were 11 genes that were differentially expressed in pancreatic cancer tissues from both the STZ and \(db/db\) mice that might participate in and play a role in promoting cancer progression. Among these genes, our study further proved that the expression of both MMP-2 and MMP-9 was abnormally upregulated in pancreatic cancer tissue under diabetic conditions. MMP-2 and MMP-9 belong to a family of MMPs known to be gelatinases that promote the degradation of type IV collagen in the basement membrane during cancer cell invasion and metastasis (60–63). In our study, when mice with pancreatic cancer were treated with a selective inhibitor of MMP-2 and MMP-9, SB-3CT, tumor volume (58) and the number of metastases significantly decreased (Figures 3D, E).

Overall, our study suggested that targeting MMP 2 and 9 is an alternative strategy that could achieve satisfactory therapeutic efficacy, as the MMP-2/9 inhibitor SB-3CT reduced malignancy, especially in terms of metastasis. In addition, in terms of tumor
size, gemcitabine was more effective than the inhibitor treatment. To combine the abovementioned advantages of using chemotherapy or inhibitors alone, an optimized therapeutic strategy was further proposed and implemented that combined an MMP-2/9 inhibitor with gemcitabine. Optimizing the therapeutic strategy significantly enhanced the antitumor effects on pancreatic cancer with concomitant diabetes and provided a basis for clinical applications.

However, the mechanism of abnormally upregulated MMP-2 and MMP-9 expression in pancreatic cancer from diabetic STZ and db/db mice was unclear. The following two possible explanations were proposed. One explanation is that diabetes induces the promotion of pancreatic cancer progression, then cancer cell frequency synthesis of MMP for adapting rapid invasion and metastasis. Alternatively, upregulated MMP-2 and MMP-9 expression might be caused by abnormal metabolic stimuli resulting from the presence of diabetes, which is known to induce the dysfunction of several intracellular signal transduction cascades; for example, protein kinase activity and the accumulation of advanced glycation end products might induce the generation of MMP overproduction under diabetic conditions (64–68). Generally, either cancer itself, diabetes, or both induced abnormal MMP-2 and MMP-9 overproduction, which should be further studied to address these unanswered questions.

**CONCLUSIONS**

Consistently, pancreatic cancer in the current study showed increased proliferation and metastatic potential in both obesity-associated and non-obesity-associated T2DM. To widen
our knowledge of diabetes as a risk factor for the development of pancreatic tumors, gene expression profiling was performed using RNA-seq. The transcriptomic analysis results showed that there were 136 and 64 DEGs in STZ and db/db mice, respectively. Among these DEGs, many are associated with carcinogenesis. This result provides useful information about potential molecular factors contributing to the malignancy of pancreatic cancer. Our results also suggest that the carcinogenesis-related genes contribute to the malignancy of pancreatic cancer are different in obesity-associated and non-obesity-associated T2DM. In addition, we found MMP-2 and MMP-9 as common therapeutic targets among 11 genes differentially expressed in both the STZ and db/db mice. These genes could be targeted to suppress the malignant progression of pancreatic cancer in both obesity-associated and non-obesity-associated T2DM. Thus, in addition to testing current therapeutic strategies against diabetes, our study offers a potential therapeutic strategy in which MMPs serve as a therapeutic target in combination with diabetes, our study offers a potential therapeutic strategy in which MMPs serve as a therapeutic target in combination with chemotherapy for diabetic pancreatic cancer patients, thus providing a basis for further study and a theoretical basis for clinical applications. In addition, the results also suggested that the choice of medication should be based on the individual's conditions; for example, the inhibitor had a lower therapeutic effect on pancreatic cancer alone; while the combination therapy may represent a better option for pancreatic cancer when diabetes is also present.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI Sequence Read Archive database (BioProject ID: PRJNA661998).

REFERENCES

1. Lopez JM, Bailey RA, Rupnow MF, Annunziata K. Characterization of type 2 diabetes mellitus burden by age and ethnic groups based on a nationwide survey. *Clin Ther* (2014) 36(4):494–506. doi: 10.1016/j.clinthera.2013.12.016
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* (2017) 60:277–301. doi: 10.3322/caac.21387
3. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* (2014) 74:2913–21. doi: 10.1158/0008-5472.CAN-14-0155
4. Giovannucci E, Harlan DM, Archer MC, Bergensdal RM, Gapstur SM, Habe LA, et al. Diabetes and cancer. *Diabetes Care* (2010) 33:1674–85. doi: 10.2337/dc10-0666
5. Rahman A. Type 2 diabetes and risk of pancreatic adenocarcinoma. *Lancet Oncol* (2014) 15:e420–0. doi: 10.1016/s1470-2045(14)70368-7
6. Shi Y, Hu FB. The global implications of diabetes and cancer. *Lancet* (2014) 383(9933):1947–8. doi: 10.1016/S0140-6736(14)60886-2
7. Coughlin SS, Calle EE, Teras LR, Petrelli J, Thun MJ. Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults. *Am J Epidemiol* (2004) 159(12):1160–7. doi: 10.1093/aje/kwh161
8. Hua F, Li K, Yu JJ, Lv XX, Yan J, Zhang XW, et al. Trb3 links insulin/igf to tumour promotion by interacting with p62 and impeding autophagic/

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee of the Medical School of Southeast University.

AUTHOR CONTRIBUTIONS

TX conceived and performed the experiments. XX and P-CL analyzed the data. Both TX and P-CL wrote the paper. SJ and HM revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.563527/full#supplementary-material
17. Huxley R, Ansary-Moghaddam A, Berrington de González A, Barzi F, Woodward M. Type II diabetes and pancreatic cancer: A meta-analysis of 36 studies. *Br J Cancer* (2005) 92:2070–6. doi: 10.1038/sj.bjc.6602619

18. Jenkins C, Elliott VL, Evans A, Oldfield L, Jenkins RE, O’Brian DP, et al. Decreased serum thrombospondin-1 levels in pancreatic cancer patients up to 24 months prior to clinical diagnosis: association with diabetes mellitus. *Clin Cancer Res* (2016) 22(7):1734. doi: 10.1158/1078-0432.CCR-15-0879

19. Aggarwal G, Ramachandran V, Javeed N, Arumugam T, Dutta S, Klee GG, et al. Adrenomedullin is up-regulated in patients with pancreatic cancer and causes insulin resistance in beta cells and mice. *Gastroenterology* (2012) 143 (6):1510–7.e1. doi: 10.1053/j.gastro.2012.08.044

20. Kikuta K, Masumune A, Hamada S, Takikawa T, Nakano E, Shimosegawa T. Pancreatic stellate cells reduce insulin expression and induce apoptosis in pancreatic beta-cells. *Biochem Biophys Res Commun* (2013) 433(3):292–7. doi: 10.1016/j.bbrc.2013.02.095

21. Gallagher EJ, Leroith D. Obesity and diabetes: the increased risk of cancer and cancer-related mortality. *Phys Rev* (2015) 95:727–48. doi: 10.1122/physrev.00030.2014

22. Yuan C, Rubinson DA, Qian ZR, Wu C, Kraft P, Bao Y, et al. Survival among patients with pancreatic cancer and long-standing or recent-onset diabetes mellitus. *J Clin Oncol* (2015) 33(29):35–39. doi: 10.1200/JCO.2014.57.5688

23. Heuson JC, Costello E, Jackson R, Halloran C, Greenhaff W, Ghanekar P, et al. The impact of diabetes mellitus on survival following resection and adjuvant chemotherapy for pancreatic cancer. *Br J Cancer* (2016) 115:887–94. doi: 10.1038/bjc.2016.277

24. Vanden Heine MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* (2009) 324:1029–33. doi: 10.1126/science.1160809

25. Yun J, Rago C, Cheong I, Pagliarini R, Cheong I, Pagliarini R, et al. Alloxan diabetes and food restriction. *Science* (2005) 309:1555–9. doi: 10.1126/science.1174229

26. Heuson JC, Legros N. Influence of insulin deprivation on growth of the 7,12-dimethylbenz(a)anthracene-induced mammary carcinoma in rats subjected to alloxan diabetes and food restriction. *Cancer Res* (1972) 32:226–32.

27. Kamisawa T, Wood LD, Itoi T, Takaori K. Pancreatic cancer. *Lancet* (2016) 388:73–85. doi: 10.1016/S0140-6736(16)00141-0

28. Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature* (2016) 531:47–52. doi: 10.1038/nature16965

29. Siravegna G, Lazzari L, Cisarolli F, Sartore-Bianchi A, Mussolin R, Casingena A, et al. Radiologic and genomic evaluation of individual metastases after 2002 blockade in colorectal cancer. *Cancer Cell* (2018) 34(1):148–62. doi: 10.1016/j.ccell.2018.06.004

30. Palazon A, Tyrikas PA, Macias D, Velic D, Rundqvist H, Fitzpatrick S, et al. An HIF-1α/VEGF-A axis in cytotoxic T cells regulates tumor progression. *Cancer Cell* (2017) 32(5):669–83. doi: 10.1016/j.ccell.2017.10.003

31. International Diabetes Foundation. *Risk Factors for Diabetes* (2005). Available at: http://www.cvd.idf.org/Diabetes/Risk_Factors_for_Diabetes/index.html (Accessed March 14, 2013).

32. Centers for Disease Control and Prevention. *Diabetes Data &Trends: Adjusted Percent age of Adults Aged 18Years or Older with Diagnosed Diabetes Who Were Overweight, United States, 1994-2010.* Available at: http://www.cdc.gov/diabetes/statistics/comp/ (Accessed March 14, 2013). Adjusted Percent age of Adults Aged 18Years or Older with Diagnosed Diabetes

33. Chou A, Fraio D, Nagrial AM, Parkin A, Murphy KJ, Chin VT, et al. Tailored first-line and second-line CDK4-targeting treatment combinations in mice models of pancreatic cancer. *Gut* (2017) 66:1–74. doi: 10.1136/gutjnl-2017-315144

34. Jones-Bosch N, Gutierrez S, Weekley A, Zhou Z, Wang C, Wang CX, et al. Compensatory metabolic networks in pancreatic cancers upon perturbation of glutamine metabolism. *Nat Commun* (2017) 8:15965. doi: 10.1038/ncomms15965

35. Lee HO, Mullins SR, Franco-Barraza J, Valianou M, Cukierman E, Cheng JD. FAP-overexpressing fibroblasts produce an extracellular matrix that enhances invasive velocity and directionality of pancreatic cancer cells. *BMC Cancer* (2011) 11(1):245. doi: 10.1186/1471-2407-11-245

36. Krüger A, Arlt MJ, Gerg M, Kopitz C, Bernardo MM, Chang M, et al. Antimetastatic activity of a novel mechanism-based gelatinase inhibitor. *Cancer Res* (2005) 65(9):3523–6. doi: 10.1181/00085472.CAN-04-3570

37. Clark CE, Hingorani SR, Mick R, Combs C, Tuveson DA, Vonderheide RH. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. *Cancer Res* (2007) 67(19):9518–27. doi: 10.1158/0008-5472.CAN-07-1075

38. Bronte V, Wang M, Overwijk WW, Surman DR, Pericle F, Rosenberg SA, et al. Apoptotic death of CDB+ T lymphocytes after immunization: induction of a suppressive population of Mac-1+/Gr-1+ cells. *J Immunol* (1998) 161 (10):5313–20.

39. Huang B, Pan PY, Li Q, Sato AL, Levy DE, Bromberg J, Rosenberg SA, et al. Tumor rejection and its potential application in Tracking Studies. *Biochem Biophys Res Commun* (2016) 475(4):484–9. doi: 10.1016/j.bbrc.2016.01.068

40. Conboy IM, Ampurdane M, Costa E, Barzi F, et al. Inhibition of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. *Immunol Rev* (2017) 280(1):93–101. doi: 10.1111/imr.12615

41. Ruotel D, Schlueter M, Coscoy L. Dysregulated cellular functions and cell stress pathways provide critical cues for activating and targeting natural killer cells to transformed and infected cells. *Immunol Rev* (2017) 280(1):93–101. doi: 10.1111/imr.12600

42. Lee HO, Mullins SR, Franco-Barraza J, Valianou M, Cukierman E, Cheng JD. FAP-overexpressing fibroblasts produce an extracellular matrix that enhances invasive velocity and directionality of pancreatic cancer cells. *BMC Cancer* (2011) 11(1):245. doi: 10.1186/1471-2407-11-245

43. Cenogorac-Jurcovic T, Effhimius E, Capelli P, Blaveti E, Baron A, Terris B, et al. Gene expression profiles of pancreatic cancer and stromal desmoplasia. *Oncogene* (2001) 20(50):7437. doi: 10.1038/sj.onc.1204935

44. Gordania B, Asuthkar S, Rao JS, Patel J, Gondi CS. Suppression of the uPAR-uPA system retards angiogenesis, invasion, and in vivo tumor development in pancreatic cancer cells. *Mol Cancer Res* (2011) 9(4):377–89. doi: 10.1158/1541-7786.MCR-10-0452

45. Roda O, Ortiz-Zapater E, Martinez-Bosch N, Gutierrez-Gallego R, Vila-Perez M, Ampurdanes C, et al. Galectin-1 is a novel functional receptor for tissue plasminogen activator in pancreatic cancer. *Gastroenterology* (2009) 136(4):1379–90. doi: 10.1053/j.gastro.2008.12.039

46. Seo Y, Baba H, Fukuda T, Takashima M, Sugimachi K. High expression of vascular endothelial growth factor is associated with liver metastasis and a
poor prognosis for patients with ductal pancreatic adenocarcinoma. Cancer (2000) 88(10):2239–45. doi: 10.1002/(sici)1097-0142(20000515)88:10<2239::aid-cncr6>3.0.co;2-v
56. Büchler P, Reber HA, Büchler MW, Friess H, Hines OJ. VEGF-RII influences the prognosis of pancreatic cancer. Ann Surg (2002) 236(6):738. doi: 10.1097/00000658-200212000-00006
57. Hart PA, Law RJ, Frank RD, Bamlet WR, Burch PA, Petersen GM, et al. Impact of diabetes mellitus on clinical outcomes in patients undergoing surgical resection for pancreatic cancer: a retrospective, cohort study. Am J Gastroent (2014) 109(9):1484–92. doi: 10.1038/aig.2014.193
58. Xu TT, Chang D, Cai Y, Min S, Ma Y, Mao H, et al. Targeting of an antecedent proteinase by an activatable probe with deep tissue penetration facilitates early visualization and dynamic malignancy evaluation of orthotopic ductal adenocarcinoma (PDAC). Biomater Sci (2019) 7(8):3320–33. doi: 10.1039/c9bm00441f
59. Scott V, Robert AW. Tumor metastasis: molecular insights and evolving paradigms. Cell (2011) 147:275–92. doi: 10.1016/j.cell.2011.09.024
60. Mu D, Cambier S, Fjellbirkeland L, Baron JL, Munger JS, Kawakatsu H, et al. The integrin alpha(v) beta8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-beta1. J Cell Biol (2002) 157:493–507. doi: 10.1083/jcb.200109100
61. Cowden Dahl KD, Symowicz J, Ning Y, Gutierrez E, Fishman DA, Adley BP, et al. Matrix metalloproteinase 9 is a mediator of epidermal growth factor-dependent e-cadherin loss in ovarian carcinoma cells. Cancer Res (2008) 68:4606–13. doi: 10.1158/0008-5472.CAN-07-5046
62. Peng YP, Zhang JJ, Liang WB, Tu M, Lu ZP, Wei JS, et al. Elevation of MMP-9 and IDO induced by pancreatic cancer cells mediates natural killer cell dysfunction. BMC Cancer (2014) 14:1–12. doi: 10.1186/1471-2407-14-738
63. Bergers G, Berge R, McMahon G, Vu TH, Itoh T, Tamaki K, et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol (2000) 2:737–44. doi: 10.1038/35036374
64. Chung AW, Hsiang YN, Matzke LA, McManus BM, van Breemen C, Okon EB. Reduced expression of vascular endothelial growth factor paralleled with the increased angiostatin expression resulting from the upregulated activities of matrix metalloproteinase-2 and -9 in human type 2 diabetic arterial vasculature. Circ Res (2006) 99:140–8. doi: 10.1161/01.RES.0000232352.90786.fa
65. Yao XM, Ye SD, Zai Z, Chen Y, Li XC, Yang GW, et al. Simvastatin protects diabetic rats against kidney injury through the suppression of renal matrix metalloproteinase-9 expression. J Endocrinol Invest (2010) 33:292–6. doi: 10.1007/BF03346588
66. Uemura S, Matsushita H, Li W, Glassford AJ, Asagami T, Lee KH, et al. Diabetes mellitus enhances vascular matrix metalloproteinase activity. Circ Res (2001) 88:1291–8. doi: 10.1161/hh1201.092042
67. Moshal KS, Tipparaju SM, Vacek TP, Kumar M, Singh M, Frank IE, et al. Mitochondrial matrix metalloproteinase activation decreases myocyte contractility in hyperhomocysteinemia. Am J Physiol Heart Circ Physiol (2008) 295:H890–897. doi: 10.1152/ajpheart.00099.2008
68. Kowluru RA, Zhong Q, Santos JM. Matrix metalloproteinases in diabetic retinopathy: potential role of mmp-9. Expert Opin Invest Drugs (2012) 21:797–805. doi: 10.1517/13543784.2012.681043

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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