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RESEARCH ARTICLE

Metadichol-Induced Differentiation of Pancreatic Ductal Cells (PANC-1) into Insulin-Producing Cells

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ABSTRACT:
Endocrine gene expression in PANC-1, a type of pancreatic cancer cell, has been studied in the context of their potential to be reprogrammed toward a normal, differentiated state. Alkaline phosphatase activity has also been shown in pluripotent stem cells to differentiate between feeder and parental cells in reprogramming experiments. Metadichol®-based cell programming holds promise as a versatile and potentially safer approach for manipulating cellular behavior without the use of viral vectors, gating, or CRISPR. Using qRT–PCR the results show multifold increase in the gene expression of *CA9*, *GCG*, *INS MAFA*, *NEUROD1*, *NGN3*, *NKX2-2*, *PAX6: PDX1*, *SLC2A2*, *FOXO1*, and *SIRT1*. ALP levels increased and this activity is often used to distinguish stem cells from feeder cells as well as from parental cells in reprogramming experiments. Pluripotency was confirmed by the presence of islet-like structures on day eight. Metadichol exhibits anticancer activity with a CC50 value of 5.50 µg/ml compared to standard doxorubicin with a CC50 value of 10.28 µg/ml. At 100 ug/ml Metadichol is 82% cytotoxic.in a MTT assay Anti-tumor gene Klotho's expression was increased 70fold on day eight. All the genes seen expressed regulate endocrine cell development in the pancreas and are involved in insulin and glucagon secretion. Gene network analysis is presented to show how Metadichol induced expression leads to a closed loop feedback network and biological process that would help in mitigating diabetes and other related disorders.

Keywords: Metadichol, PANC-1, Gene expression, beta cells, Glut2, Nuclear receptors, reprograming cancer cells, Diabetes, Insulin, Glucagon, Alkaline phosphatase, Klotho

Introduction

Diabetes mellitus is a widespread chronic condition that has no cure despite numerous treatment options. However, inducing the regeneration of insulin-producing cells in the pancreas through regenerative medicine has emerged as a promising approach for treating diabetes.[1] Accordingly, regenerative therapy offers several advantages over conventional treatment; it aims to restore normal insulin secretion, potentially leading to a cure for diabetes.[2] Furthermore, it can reduce the risk of complications associated with diabetes, such as cardiovascular disease, neuropathy, and retinopathy.

Pancreatic carcinoma cell line (PANC1) can transdifferentiate into endocrine precursors through neurogenin 3 (NGN3) activation. Recent studies have shown that human fibroblast growth factor (FGF)-2b and human placental lactogen (hPL)-A can induce an increase in PANC-1 duct cell dedifferentiation into islet-like aggregates.[3] Compared with control cells, these clusters of transcription factors have typical ultra-structures of islet aggregates and can induce glucose homeostasis by increasing the secretion of insulin, C-peptide, pancreatic and duodenal homeobox 1 (PDX1), NK2 homeobox 2 (NKX2-2), glucagon (GCG), and glucose transporter protein 2 (GLUT2). However, several challenges must be addressed before clinical application, such as the long-term safety and efficacy of the therapy, in addition to the manner of delivery to the pancreas, to induce pancreatic stem cell differentiation into insulin-producing cells and factors such as the encapsulation and transplantation of islet-like clusters.

Table 1 is a summary of the functions and pathways associated with genes mainly involved in the regulation of insulin secretion and glucose metabolism, particularly in the pancreas.[4,5]. Endocrine genes encode hormones or hormone receptors that regulate various physiological processes in the body are involved in the development and function of pancreatic beta (β) cells that produce insulin are *NEUROG3*, *INS*, *SLC2A2*, and *MAFA*. Treatment with a combination of small molecules targeting specific signaling pathways could induce differentiation in PANC-1 cells, leading to changes in endocrine gene expression.[6,7,8]

Table 1: List of genes involved in insulin secretion and glucose metabolism

Gene	Encoding	Pathway roles
CA9	Carbonic anhydrase IX	pH regulation in pancreatic islet β-cells and insulin secretion
GCG	Glucagon (produced by α-cells of the pancreatic islets of Langerhans)	Regulation of glucose homeostasis by stimulating glucose production in the liver
INS	Insulin (produced by β-cells of the pancreas)	Regulation of glucose metabolism by stimulating glucose uptake from the blood into tissue cells
MAFA	MAF bZIP A transcription factor	β-cell development and function
NEUROD1	Neuronal differentiation 1, a Transcription factor	Pancreas development and insulin secretion regulation
NEUROG3	Neurogenin 3 a Transcription factor	Endocrine cell development in the pancreas, including β-cells
NKX2-2	NK2 homeobox 2 a Transcription factor	β-cell development and function
PDX1	Paired Box 6, a transcription factor	β-cell development and function
SLC2A2	Glucose transporter protein (GLUT2), primarily expressed in pancreatic β-cells	Insulin secretion regulation by importing glucose into the pancreas
FOXO1	Forkhead box O1 A Transcription factor	Glucose metabolism regulation and insulin sensitivity
SIRT1	Sirtuin 1, a transcription factor	Glucose metabolism regulation and insulin sensitivity

However, reprogramming pancreatic cancer cells presents many challenges;[9-11]. Dong[12] explored the potential for reprogramming the tumor microenvironment in pancreatic cancer, including endocrine gene expression, to improve glucose metabolism and decrease tumor growth.[13]. Ghani et al.[14] investigated the effect of BMP-7 protein on PANC-1 cells and found that when insulin expression increased, glucose-stimulated insulin secretion improved, and the expression of the genes involved in β-cell differentiation and function changed, suggesting that BMP-7 ((Bone morphogenetic protein

7)) treatment induces the differentiation of PANC-1 cells toward a more β-cell-like phenotype.

Additionally, Pedica et al.[15] showed that PDX1, the endocrine transcription factor PDX1, is expressed in the pituitary gland and can regulate the expression of pituitary hormones. The study used PANC-1 cells as a model for investigating PDX1 function.[16] Conversely, Ebrahim et al.[17] showed that PDX1 is critical for maintaining the secretory function of the duodenum, and PANC-1 cells were used to investigate the role of PDX1 in regulating gene expression in a part of the small intestine.

Nishinura [18] reviewed the role of MAFA transcription factors in pancreatic β-cells while discussing their potential use to improve insulin secretion in pancreatic cancer cells such as PANC-1. The mechanism involves FGF-2b/hPL-A inducing the differentiation of PANC-1 cells, which are known to express some endocrine genes when stimulated by cytokines or other factors, suggesting a potential method for ductal-to-endocrine trans differentiation. We used Metadichol®[19] to treat PANC-1 cells, evaluated the increased expression of the genes in question and morphologically characterized them for the presence of islets.

Methods

SAMPLE PREPARATION AND RNA ISOLATION:

The treated PANC-1 cells were dissociated and rinsed with sterile 1× PBS solution and then centrifuged. We removed the supernatant and added 0.4 mL of TRIzol and gently mixed for 1 min. The samples were maintained at room temperature for 10 min, and 0.25-mL chloroform per 0.4-mL TRIzol was used and mixed in a vortex for 15 s. After 5 min, we centrifuged the mixture at 12,000 rpm for 15 min at 4°C. The upper aqueous phase was collected in a new sterile microcentrifuge tube to which we added 0.5 mL of isopropanol, with gentle inversions for 30 s and incubation at −20°C for 20 min. Next, we centrifuged at 12,000 rpm for 10 min at 4°C. We removed the supernatant and washed the RNA pellet by adding 0.5 mL of 70% ethanol. The RNA mixture was centrifuged at 12,000 rpm at 4°C, the supernatant was carefully discarded, and the pellet was air-dried. Then, the pellet was resuspended in 20 µL of DEPC-treated water, and we quantified the total RNA yield using Spectradrop (Spectramax i3x, Molecular Devices, USA) (Table 2).

Table 2: Total RNA yield

Treatment Group	Treatment	RNA yield (ng/µL)
Control	-	350.56
Sample	1 ng	264.72
	100 ng	390.56
	1 pg	388.08
	1 pg	388.08
	100 pg	343.12

cDNA SYNTHESIS

We synthesized cDNA from 500 ng of RNA using the cDNA synthesis kit from the Prime Script RT reagent kit (TAKARA) with oligo dT primer following the manufacturer's instructions. We set the reaction volume at 20 µL and synthesized cDNA at 50°C for 30 min, followed by RT inactivation at 85°C for 5 min using the applied biosystem Veritii. The cDNA was further used for real-time PCR analysis.

PRIMERS AND qPCR ANALYSIS

The PCR mixture (20 µL) contained 1.4 µL of cDNA, 10 µL of SYBR Green Master mix, and 1 µM of the respective complementary forward and reverse primers (Table 3) specific for the respective target genes. Enzyme activation was conducted at 95°C for 2 min, followed by a two-step reaction with an initial denaturation and annealing cum extension step at 95°C for 5 s, annealing for 30 s at the appropriate respective temperature amplified for 39 cycles, and a secondary denaturation at 95°C for 5 s and 1 cycle with a melt curve capture step ranging from 65°C to 95°C for 5 s each. We analyzed the obtained results and calculated the fold expression.

Table 3: Primer details; Target specific forward and reverse primers

Primer	Sequence	Annealing temperature (°C)
GAPDH	GTCTCCTCTGACTTCAACAGCG	60
	ACCACCCTGTTGCTGTAGCCAA	
GLUT2	ATGTCAGTGGGACTTGTGCTGC	61
	AACTCAGCCACCATGAACCAGG	
CA9	CACTCCTGCCCTCTGACTTC	60
	TCGGAAGTTCAGCTGTAGCC	
PAX6	CTGAGGAATCAGAGAAGACAGGC	58
	ATGGAGCCAGATGTGAAGGAGG	
Glucagon	TTCCCAAAGAGGGCTTGCTC	58
	TGCCTGGGAAGCTGAGAATG	
Insulin	ACGAGGCTTCTTCTACACACCC	60
	TCCACAATGCCACGCTTCTGCA	
PDX1	GAAGTCTACCAAAGCTCACGCG	60
	GGAACTCCTTCTCCAGCTCTAG	
NEUROD-1	GGTGCCTTGCTATTCTAAGACGC	60
	GCAAAGCGTCTGAACGAAGGAG	
NKX2.2	CCTTCTACGACAGCAGCGACAA	63
	ACTTGGAGCTTGAGTCCTGAGG	
MAFA	GCTTCAGCAAGGAGGAGGTCAT	60
	TCTGGAGTTGGCACTTCTCGCT	
NGN3	CCTAAGAGCGATTGGCACTGA	60
	AGTGCCGATTGAGGTTGTGCA	
Sirtuin 1	TAGACACGCTGGAACAGGTTGC	57
	CTCCTCGTACAGCTTCACAGTC	
FOXO1	CGCCACATTCAACAGGCAG	60
	CCATCCACATCGAGGCTCC	

CELL CULTURE AND MAINTENANCE

Briefly, we cultured the PANC-1 cells (CRL-1469) in DMEM complete media supplemented with 10% FBS and 1% Penstrep in a humidified atmosphere of 5% CO_2 at 37°C with frequent media changes every other day until confluency was achieved. We assessed cell viability by staining with trypan blue using a hemocytometer. An appropriate cell density will be further seeded to expand the cell lines for further studies.

CELL SEEDING

The cell density at 0.2 × 06 cells/mL of media was seeded into 6-well plates and incubated for 24 h at 37°C with 5% CO_2 to assess the effects of Metadichol®.

CELL TREATMENT

After 24 h of seeding, the medium was carefully removed, and cells were incubated with differentiation medium supplemented with DMEM-F12 nutrient mixture (Gibco) along with various concentrations of Metadichol® (Table 4) and incubated at 37°C with 5% CO_2 for an appropriate period of 8 days. Afterward, we harvested the cells and determined ALP activity. During the treatment, we recorded images of the cells every other day for 8 days.

Table 4: Treatment concentrations

Sample	Treatment	Cell line
Control	0	PANC-1 (ATCC CCL-1469)
Sample	1 ng	
	100 ng	
	1 pg	
	100 pg	

ALP ASSAY

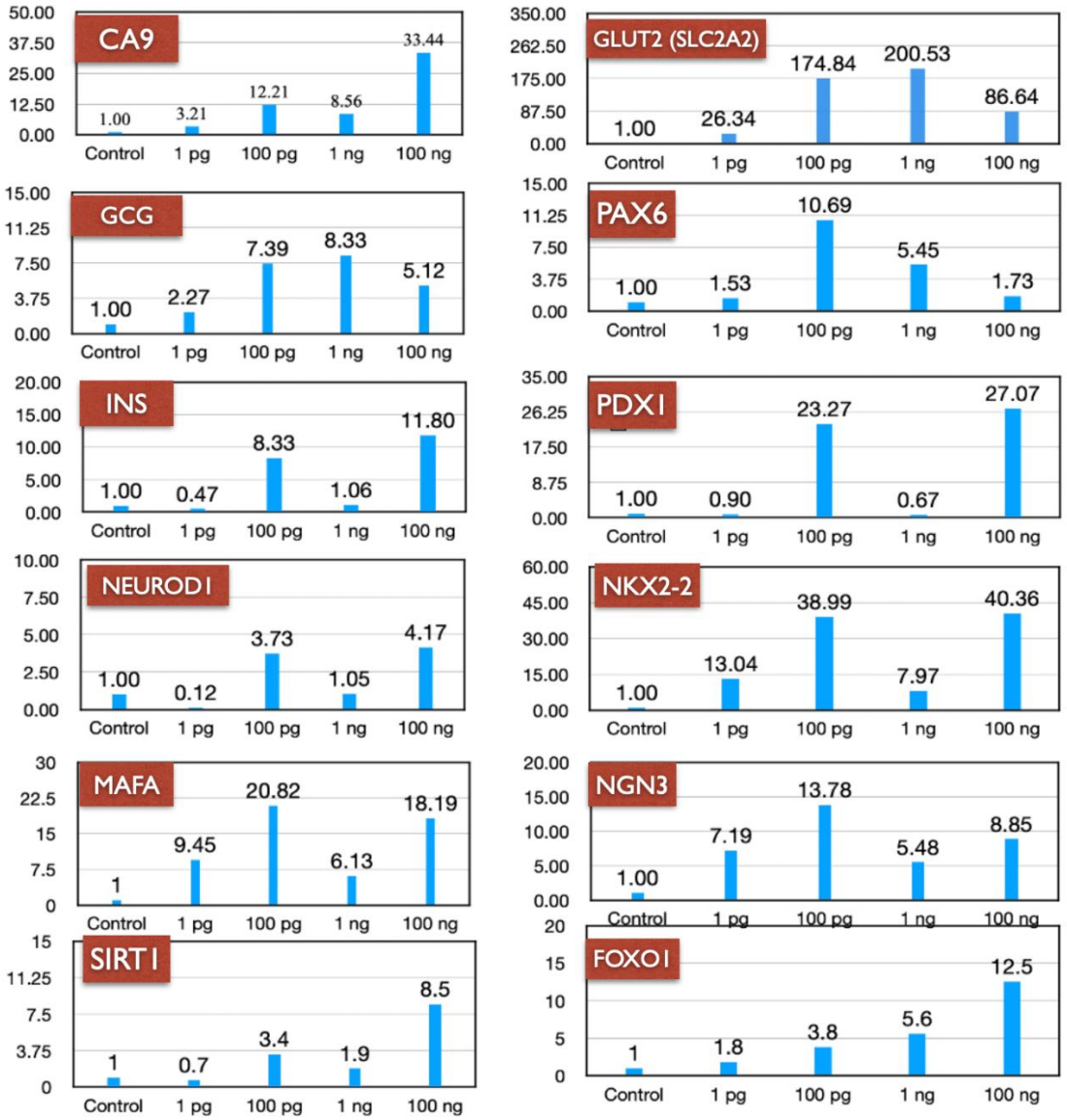
We completely isolated the protein by suspending the harvested cells in 200 µL of lysis buffer containing PMSF. ALP assay master stock containing AMP and pNPP substrate (2.5 mM) was prepared. A 10 µL of protein sample and 100 µL of master stock was added to this and incubated at 37°C for 3 min, and the kinetic measurements were recorded at 405 nm, followed by determination of ALP activity using the following formula:

ALP activity (U/L) = ΔA/min ∗ 2720.

Results

All targeted genes involved in endocrine differentiation were all overexpressed. Most impressive was *SLC2A2* (GLUT2) increased by >200-fold and PDX1 increased 27-fold. There was in 70-fold increase in Klotho expression on day 8. Alkaline phosphatase (ALP) activity is often used to distinguish stem cells from feeder cells as well as from parental cells in reprogramming experiments (Figure 2) was significantly increased when Metadichol @100 pg/m was used. We also tested metadichol at higher concentrations using doxorubicin as a standard and carried out a MTT assay as a means of measuring the activity of living cells via mitochondrial dehydrogenases[20], See Table (6). A lower CC50 value indicates higher anticancer activity. Metadichol exhibits anticancer activity with a CC50 value of 5.50 µg/ml compared to standard doxorubicin with a CC50 value of 10.28 µg/ml. At 100 ug/ml Metadichol is 82% cytotoxic to the cells.

Figure 1: qRT-PCR-Fold increase



Discussion

Glucose transporter Glut2 that is expressed in pancreatic β-cells plays a key role in glucose-stimulated insulin secretion by sensing changes in blood glucose levels.[21] The cells are known to show a multifold increase in GLUT2 expression when treated with metformin, a drug that lowers blood glucose levels by inhibiting hepatic gluconeogenesis.[22] The relevance of this increase could be related to the role of GLUT2 in regulating intracellular glucose metabolism and the sensitivity to apoptosis in PANC-1 cells. Metformin induces GLUT2 expression through AMPK activation, and GLUT2 mediates the antiproliferative and proapoptotic effects of metformin on PANC-1 cells.[23]

Homeobox protein *NKX2-2* expression is regulated in PANC-1 cells by PDX1,[24] showed a 40-fold increase. PDX1 binds and activates the transcription of the *NKX2-2* promoter and can cooperate with NEUROD1, a basic helix–loop–helix transcription factor that is critical for β-cell maturation and maintenance, to enhance *NKX2-2* expression.[25]. This suggests that PDX1 and NEUROD1 overexpression in PANC-1 cells leads to increased *NKX2-2* expression and induces a more β-cell-like phenotype.

Pancreatic cell interconversions depend on NGN3, a key endocrine progenitor transcription factor necessary for the specification of endocrine cells, and lead to differentiation into insulin-producing cells in ductal progenitor cells.[26,27]. Results indicate a 13-fold increase in *NGN3* expression in PANC-1 cells. Notch signaling is a conserved pathway that controls cell fate decisions and maintains progenitor cells in an undifferentiated state. It also represses *NGN3* expression by inhibiting the activity of PDX1.[28] *Sirtuin1* expression is increased by 10-fold. Notch signaling represses *SIRT1* expression by inhibiting the activity of NGN3, which can bind to the promoter of *SIRT1* and activate its transcription.

There is a low expression of *PAX6* compared with normal pancreatic ductal cells; therefore, a 10-fold expression of *PAX6* is significant. A study found that *PAX6* overexpression in PANC-1 cells inhibits their growth, migration, and invasion by inducing apoptosis and cell cycle arrest.[29] The significance of increased *PAX6* expression in PANC-1 cells could be related to its role in regulating the differentiation and function of pancreatic endocrine cells, including alpha (α) cells that produce glucagon and β-cells that produce insulin. It is likely that *PAX6* may act as a tumor suppressor in pancreatic cancer by restoring the normal phenotype and function of pancreatic ductal cells.[30]

There is an increase of 20- and 4fold, of *MAFA* and *NEUROD1* expression respectively, and they synergistically activate the *SLC2A2* (GLUT2) gene in β-cells.[31] Insulin gene expression and β-cell function is regulated by MAFA and one of the key factors that defines the mature β-cell phenotype, along with PDX1.[32] Under normal conditions, PANC-1 cells do not express insulin or other β-cell markers. Generally, the expression of *MAFA* is very low or absent and its expression can be induced in PANC-1 cells by various factors, such as PDX1, a transcription factor that is essential for pancreatic development and β-cell function and can activate the MAFA promoter and increase the mRNA levels in PANC-1 cells.[33]

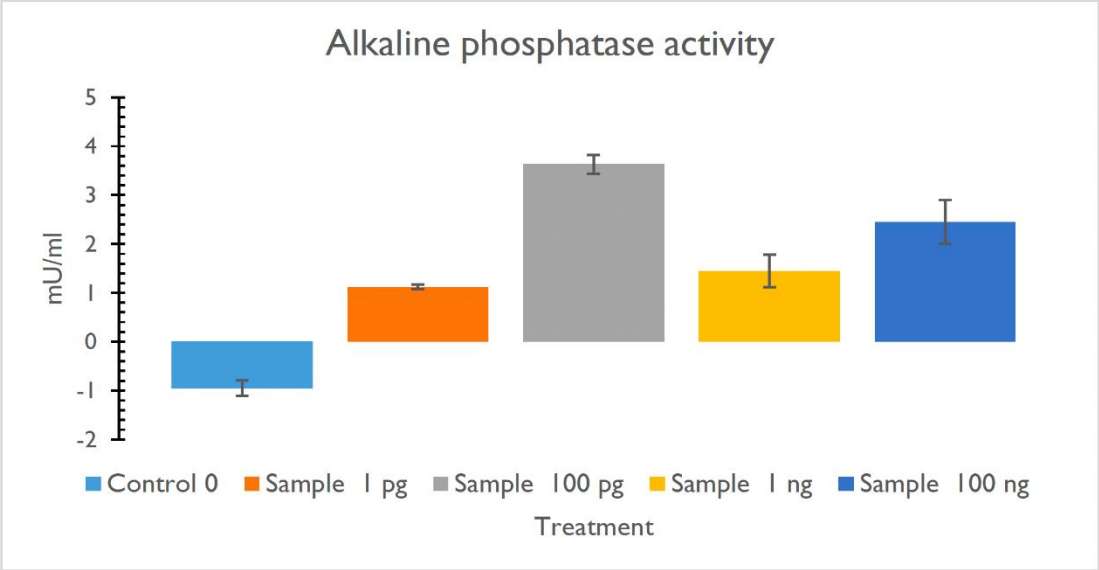
The results show overexpression of *FOXO1* in PANC-1 cells, and this can suppress proliferation, migration, and invasion by inducing apoptosis and cell cycle arrest.[34] Forkhead box O1 (FOXO1) is known to respond to glucose levels and insulin receptor activation by modulating the expression of genes involved in glucose uptake, glycolysis, gluconeogenesis, and glycogen synthesis. The significance of increased *FOXO1* expression in PANC-1 cells could be related to its role in regulating the differentiation and function of pancreatic endocrine cells, including α-cells that produce glucagon and β-cells that produce insulin.[35] Thus, *FOXO1* may act as a tumor suppressor in pancreatic cancer by restoring the normal phenotype and function of pancreatic ductal cells.

Alkaline phosphatase (ALP) activity (Figure 2) was significantly increased when Metadichol @100 pg/m was used. ALP activity is upregulated in pluripotent stem cells, including undifferentiated embryonic stem and germ cells and induced pluripotent stem cells.[36] ALP activity is often used to distinguish stem cells from feeder cells as well as from parental cells in reprogramming experiments. This confirms its pluripotency through the presence of islet-like structures on day 8 (Figures 3 and 4).

It is unlikely that Metadichol ® alone can induce expression of all the discussed genes and or transcription factors. Up field genes are likely to be activated by binding with Metadichol® in a cascade of signaling events that lead to the 12 expressed set of genes/ transcription factors.. We recently showed that Metadichol® can activate all 48 nuclear receptors (NRs) in human mesenchymal stem cells (HMSCs) at concentrations ranging between 1 pg/mL and 100 ng/mL.[37] Therefore, downfield gene expression could result from NR activation.

Figure 2: ALP activity in cells treated with Metadichol

		ALP Activity (mU/ml)			
Sample	**Concentration**	**n=1**	**n=2**	**n=3**	**Mean ± SD**
Control	**0**	-1.09	-1.00	-0.77	-0.95 ± 0.16
Metadichol	**1 pg**	1.09	1.18	1.09	1.12 ± 0.05
	100 pg	3.45	3.63	3.82	3.63 ± 0.19
	1 ng	1.54	1.72	1.09	1.45 ± 0.33
	100 ng	0.45	0.82	0.73	2.24 ± 0.45



For example, retinoic acid, a metabolite of vitamin A, can bind to nuclear retinoic acid receptors (RARs) and modulate the expression of *PAX6* and other genes involved in eye development.[39] RAR-NRs can also regulate the expression of PDX1, NKX6.1, and MAFA transcription factors that are crucial for β-cell development and maturation by interacting with their promoters.[40] Another example is NR4A1, which can bind to the MAFA promoter and enhance its expression in pancreatic β-cells.[41] Additionally, NR4A1 can bind to the CA9 promoter and enhance its expression under hypoxic conditions, and it can activate the expression of insulin and other genes involved in glucose homeostasis. Conversely, NR4A2 can bind to the GCG promoter and enhance its expression in pancreatic α-cells[42] and to the NEUROD1 promoter and enhance its expression in dopaminergic neurons. A 27-fold increase seen for PDX1 which is known to enhance GLUT2 expression in PANC-1 cells. NR4A2 a transcription factor is essential for the development and function of the liver and pancreas, can bind to the promoter of GLUT2 and activate its transcription.[42]Additionally, it can cooperate with PDX1, another homeobox transcription factor that is essential for pancreatic development and β-cell function.[43]

Figure 3: Differentiation of PANC-1 treated with Metadichol® by morphological characterization.

PANC-1, pancreatic ductal cells

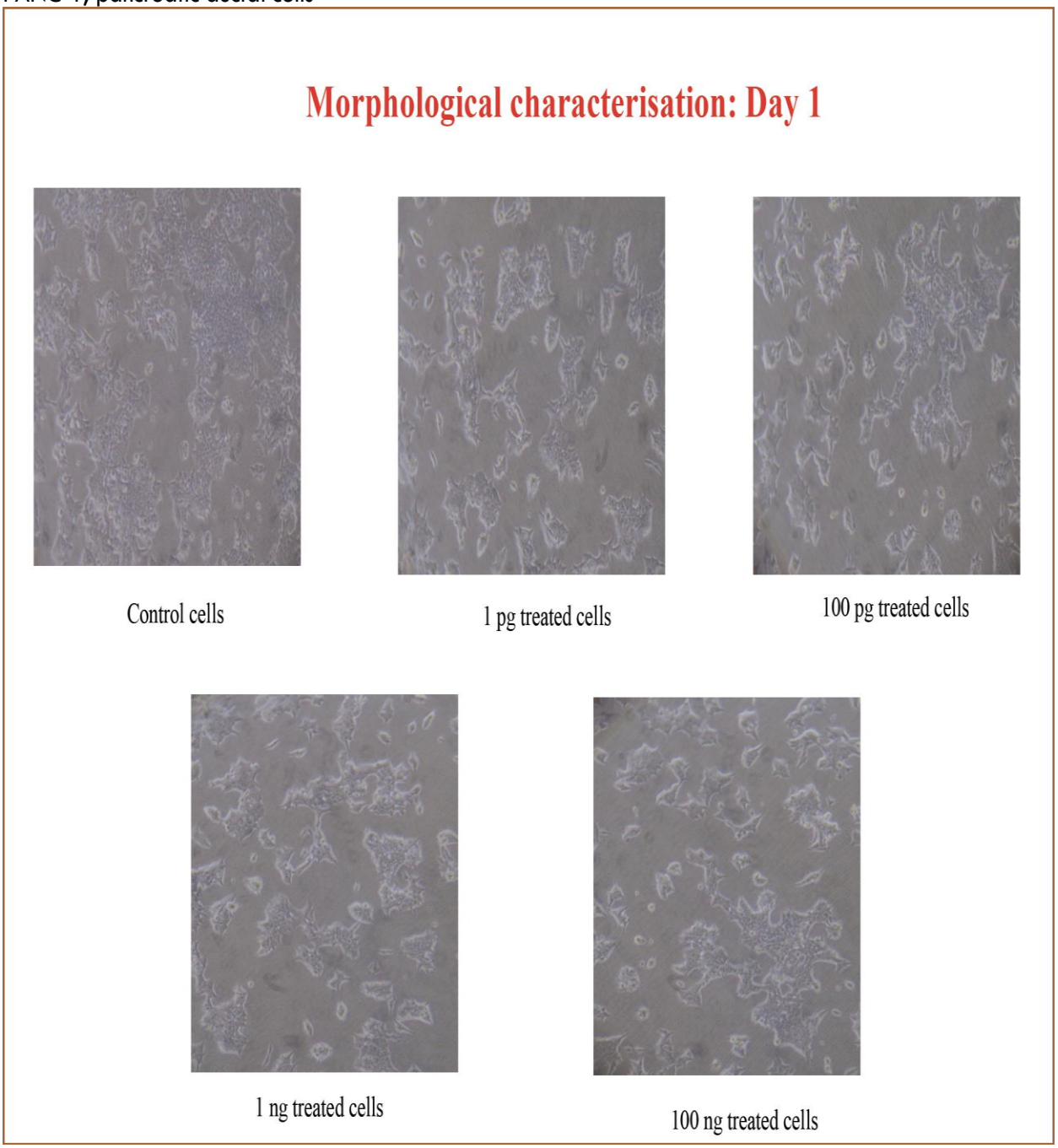
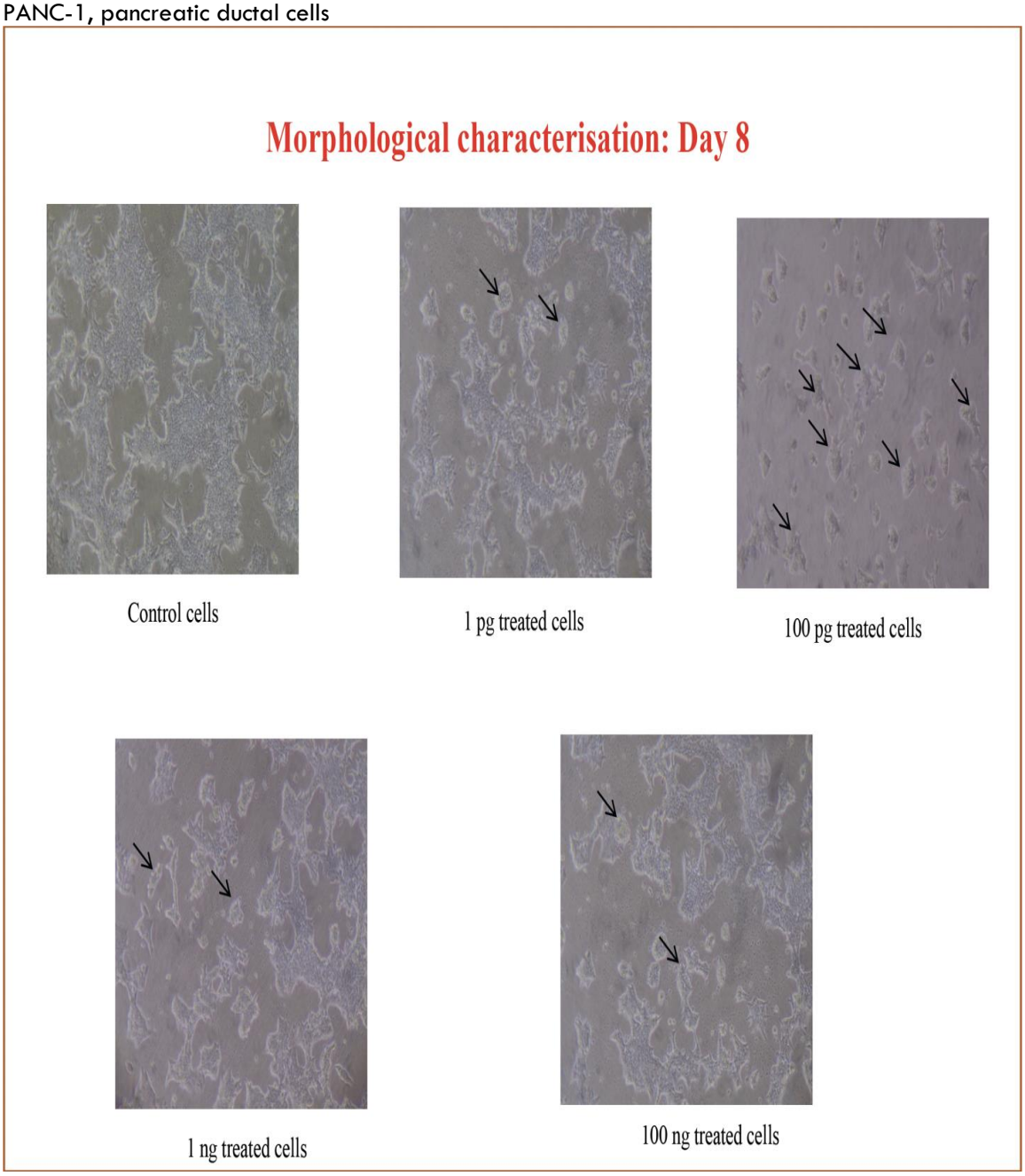


Figure 4: Differentiation of PANC-1 cells into β-cells at day 8

Black arrows indicate the formation of ILCC
ILCC, islet-like cell clusters
PANC-1, pancreatic ductal cells



NR5A2 can bind to the PAX6 promoter and enhance its expression in pancreatic β-cells.[44] It can also activate the expression of insulin and other genes involved in glucose homeostasis in pancreatic β-cells. Similarly, NR5A2 can bind to the NKX2-2 promoter, enhance its expression in pancreatic endocrine progenitor cells, and activate the expression of *MAFA* and other genes involved in β-cell maturation.[45] Furthermore, NR5A2 activates the expression of PDX1, NKX2-2, and NGN3, transcription factors that are involved in endocrine cell differentiation.[46] The liver X receptor (LXR) is an NR that regulates cholesterol homeostasis, lipid metabolism, and inflammation. LXR can activate SIRT1 by increasing its expression or by directly interacting with it and stimulating its deacetylase activity. SIRT1 can also deacetylate and activate LXR, creating a positive feedback loop.[47-49]

Thyroid hormone (TH), a metabolite of the thyroid gland, can bind to nuclear TH receptors (THRA and THRB) and modulate and regulate the expression of PDX1, another transcription factor that is crucial for pancreatic β-cell development and function,[50] by interacting with MAFA on the PDX1 promoter.[51]

Table 5: NR expression in Stem cells

Nomenclature name	Common name	1 pg	100 pg	1 ng	100 ng	Control
NR1A1	THRA	**16.16**	12.24	7.7	5.32	1
NR1A2	THRB	**7.71**	**1.94**	**15.11**	**8.71**	1
NR1B1	RARA	1.27	0.79	0.52	0.44	1
NR1B2	RARB	**1.67**	1.39	0.48	0.73	1
NR1B3	RARG	**2.52**	1.04	0.96	0.82	1
NR1H3	LXRA	1.28	0.97	0.55	0.19	1
NR1H2	LXRB	1.28	1.17	0.84	0.18	1
NR4A1	NGFIB	**1.82**	0.67	1.16	0.61	1
NR5A2	LRH1	1.3	0.72	0.29	0.15	1
NR4A2	NURR1	0.48	4.55	5.24	7.44	1

All these relevant NRs (nuclear receptors) are expressed by stem cells treated with Metadichol®, as shown in Table 5. Our study adds some additional information and shows for the first time that all the genes needed for differentiation are directly activated by upstream genes involved in transcription, namely, NRs. An increased number of involved NRs leads to a high degree of regulation. These NRs express all the downstream genes needed for differentiation. The key is the activation of NRs by Metadichol®, leading to a tight set of highly connected genes. These genes have more interactions among themselves than expected for a gene set of the same size and distribution degree randomly selected from the genome. This enrichment indicates that this set of genes shares a significant biological connection. The analysis of gene networks Pathway Studio and protein–protein interaction maps[52-54] indicated the formation of a loop feedback network, as shown in Figure 5.

Metadichol induces expression of Klotho, an antitumor molecule that controls the growth of cancer cells.[55,56] We also measured Klotho expression and showed a seventy-fold increase after 8 days (Table 6 and Figure 6).

Table 6: Primer details; Target specific forward and reverse primers

KLOTHO	Forward GTGCGTCCATCTGGGATACG
	Reverse TGTCGCGGAAGACGTTGTT

Sample	Mean Cq	Relative Fold Expression
Control	25.25	1.00
1 pg	24.65	12.86
100 pg	24.17	51.87
1 ng	23.16	24.41
100 ng	23.76	68.94

Figure 5: Protein-Protein Interaction maps

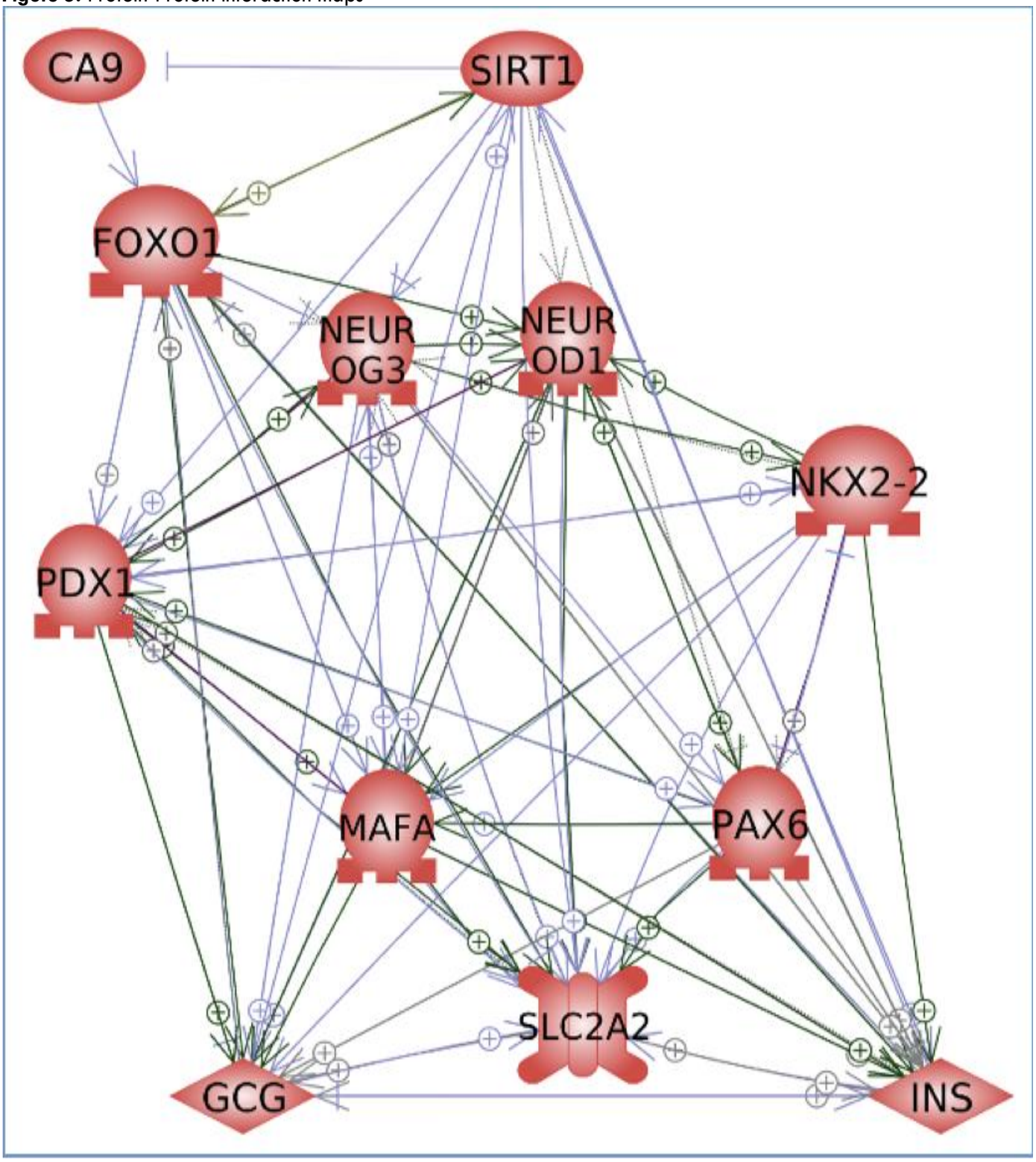
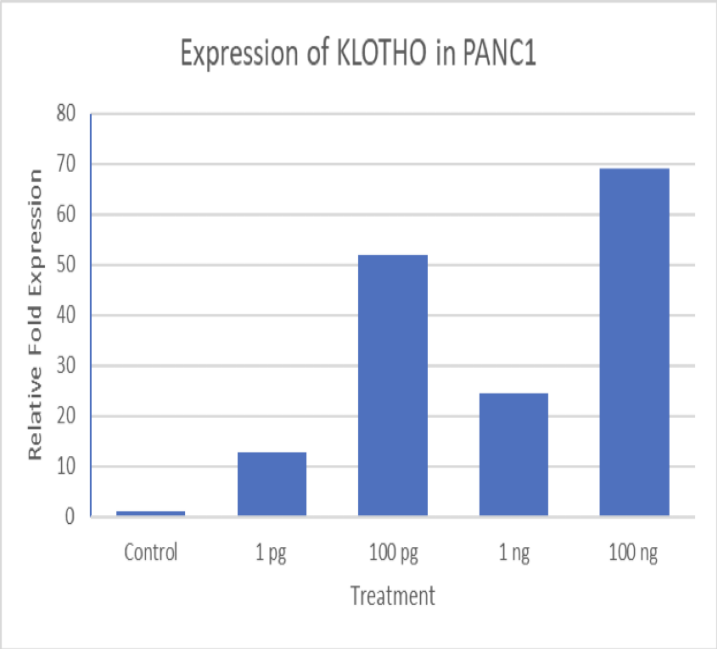


Figure 6: Normalized expression of KLOTHO gene



An analysis of gene networks was performed using Pathway Studio software and showed a closed loop feedback network (Figure 5). All the important processes (table 7) are related to β-cells, α-cells, and insulin synthesis. A complete list of cellular processes regulated by expressed genes is available in the Supplementary Material.

NEUROD1, NEUROG3, NKX2-2, PAX6, PDX1, and MAFA are all transcription factors that play critical roles in the development and function of pancreatic β-cells, which produce and secrete insulin. These factors can activate or repress the expression of each gene, and their interaction is essential for the proper development and function of β-cells.

Glucose transporter SLC2A2 (GLUT2) is expressed in pancreatic β-cells and plays a role in regulating insulin secretion. The expression of SLC2A2 is regulated by transcription factors such as PDX1 and FOXO1. Hormones INS and GCG produced by pancreatic islet cells play opposing roles in the regulation of glucose metabolism. Insulin promotes the uptake and storage of glucose, whereas glucagon promotes the release of glucose into the bloodstream. Various transcription factors, including PDX1, MAFA, and FOXO1, regulate INS and GCG expression. pH in pancreatic cells is regulated by CA9, which is essential for proper insulin secretion.

Both SIRT1 and FOXO1 are involved in regulating glucose metabolism and insulin sensitivity. SIRT1 deacetylates and activates FOXO1, which can promote the expression of genes involved in glucogenesis and insulin resistance. However, under certain conditions, such as calorie restriction, when intracellular NAD+ levels are high, SIRT1 can deacetylate and inhibit FOXO1, improving glucose homeostasis and insulin sensitivity. This effect is thought to be mediated through several mechanisms, including the promotion of mitochondrial biogenesis, the activation of AMPK, and the repression of genes involved in gluconeogenesis and inflammation. Some studies have suggested that SIRT1-mediated inhibition of FOXO1 plays a role in the beneficial effects of caloric restriction and fasting on glucose metabolism and lifespan.

Table 7: Top 10 list of significantly enriched pathways. (The complete list is in the Supplementary Material)

Name	Overlap	Overlap %	p value	Genes from the list
Transcription factors in β-cell neogenesis (rodent model)	7	28	$1.25 (\times) 10^{-16}$	*NKX2-2 PAX6, PDX1,NEUROG3,MAFA, INS, NEUROD1.*
α-Cell to β-cell interconversion (hypothesis)	6	27	$3.64 (\times) 10^{-14}$	*PAX6, PDX1, GCG, NEUROG3, INS, NEUROD.1*
Neonatal diabetes mellitus	6	19	$3.58 (\times) 10^{-13}$	*PAX6, PDX1, NEUROG3; SLC2A2; INS; NEUROD1*

Name	Overlap	Overlap %	p value	Genes from the list
FOXO1 and SREBP-1C roles in β-cell suppression (rodent model)	6	14	$2.54 (\times) 10^{-12}$	*SIRT1, PDX1, MAFA; INS; NEUROD1, FOXO1*
Insulin synthesis in β-cell	6	11	$9.79 (\times) 10^{-12}$	*SLC2A2, PAX6; PDX1; MAFA; INS; NEUROD1*
β-Cell death in diabetes mellitus type 2	5	13	$2.84 (\times) 10^{-10}$	*PDX1, MAFA, SLC2A2, INS; NEUROD1*
Insulin secretion	5	13	$3.78 (\times) 10^{-10}$	*PDX1, GCG, SLC2A2, INS; NEUROD1*
Autoimmune polyglandular syndrome progression (hypothesis)	3	21	$4.35 (\times) 10^{-7}$	*PDX1, INS, MAFA*
Maturity-onset diabetes of the young (MODY)	3	16	$9.72 (\times) 10^{-7}$	*PDX1, INS;,NEUROD1*
GLIS3 targets in thyroid dysgenesis (hypothesis)	3	13	$2.1 (\times) 10^{-6}$	*PAX6, MAFA, INS*

Metadichol®, as we have shown, here can reprogram cancer cells at very low concentrations. and toxic at higher concentrations to cells, leading to cell death (Table 8). This can be beneficial if the goal is to eliminate cancer cells. (Full Experimental in supplementary files).

Table 8: Cytotoxicity of test compounds in Panc1 cells

Panc1		n = 1		n = 2		Average % Cytotoxicity	SD	CC50 (µg/mL)
Sample	Conc.(µg/mL)	Abs at 590nm	% Cytotoxicity	Abs at 590nm	% Cytotoxicity			
Control	0	0.764	0.0	0.749	0.0	0.00	0	
Metadichol	0.14	0.775	-1.45	0.749	-0.01	0.00	0.00	5.5
	0.41	0.718	6.01	0.718	4.10	5.06	1.36	
	1.23	0.639	16.32	0.618	17.47	16.90	0.81	
	3.70	0.496	35.16	0.503	32.77	33.97	1.69	
	11.11	0.345	54.85	0.348	53.54	54.20	0.93	
	33.33	0.284	62.81	0.255	65.90	64.36	2.18	
	100.00	0.141	81.57	0.134	82.07	81.82	0.36	

Doxorubicin		n = 1		n = 2		Average % Cytotoxicity	SD	CC50 (µg/mL)
Sample	Conc.(µg/mL)	Abs at 590nm	% Cytotoxicity	Abs at 590nm	% Cytotoxicity			
Control	0	0.764	0.0	0.749	0.0	0.00	0	
Doxorubicin	1.70	0.690	9.8	0.669	10.7	10.22	0.32	10.28
	3.40	0.606	20.7	0.591	21.1	20.90	0.12	
	6.79	0.493	35.5	0.501	33.2	34.32	0.83	
	13.59	0.329	56.9	0.323	56.9	56.88	0.02	
	27.18	0.241	68.4	0.219	70.8	69.62	0.83	
	54.35	0.146	80.9	0.119	84.2	82.56	1.14	

The lower CC50 value indicates higher anticancer activity. Metadichol exhibits anticancer activity with a CC50 value of 5.50µg/mL compared with the standard doxorubicin with a CC50 value of 10.28µg/mL. Metadichol is 82% cytotoxic at a concentration of 100 ug/mL. The gap between the reprogramming and toxic concentrations is preferred as it provides a safety margin for treatment. The differential effects of compounds at various concentrations can lead to personalized medicine in which the dose can be tailored to an individual patient's needs. Reprogramming instead of outright killing reduces the chances of a tumor developing drug resistance. Metadichol can be combined with other therapies to enhance the overall efficacy. For instance, reprogramming can be followed by traditional chemotherapeutics to eliminate the now "normalized" cancer cells.

Another molecule that can enhance endocrine differentiation of Panc1 cells is vitamin C, which can enhance the endocrine differentiation of human embryonic stem cells (hESCs) by increasing the expression of key transcription factors, such as PDX1, NKX6.1, and MAFA.[57] Vitamin C can thus improve the efficiency and quality of iPSC formation by activating pluripotency genes and erasing epigenetic memory in the adult cell state.[58] We have published work showing how Metadichol increases Vitamin C in vivo in a human study [59-61]

Conclusions

Metadichol® simultaneously increased the expression of insulin, glucagon, and SLC2A2 in PANC-1 cells, which has potential implications.

Blood sugar regulation

Increasing the expression of insulin and glucagon, along with SLC2A2, in PANC-1 cells regulates blood sugar levels. Insulin helps lower blood sugar, glucagon raises it, and SLC2A2 facilitates glucose uptake into cells. This could have implications for the metabolism of both cancer cells and the surrounding tissues.

Cancer cells

Manipulating the expression of insulin and glucagon in cancer cells could have complex effects on the tumor's biology. Insulin has been shown to have growth-promoting effects, which might raise concerns about increased cancer cell proliferation. Conversely, the role of glucagon in stimulating the release of glucose from the liver might have implications on tumor metabolism.

Therapeutic strategy

This induction of the expression of both insulin and glucagon in cancer cells might be explored as a novel therapeutic strategy because Metadichol® is a nontoxic molecule and safe for human use.[62-64]

Metabolic control

Manipulating the hormonal environment within cancer cells might have broader metabolic effects beyond blood sugar regulation. This will affect other cellular processes and signaling pathways

This research shows that diseases, pathways and biological processes are closely connected through related gene networks, and this is an approach that can be exploited to modulate multiple targets to enhance therapeutic effects, as ligands today are focused on a single target and limited by their efficacy. Metadichol® represents the first of a safe class of therapeutics that target multiple genes, pathways [65-68] and multiple diseases which points to the importance of our results to a new approach for mitigating disease.

Conflicts of interest statement: The authors have no conflicts of interest to declare.

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Data availability: All raw data are presented in the Supplementary Material.

Supplementary material: Raw Data q-RT–PCR; Gene list network analysis, MTT assay experimental Raw data

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