Journal of Yoga, Physical Therapy and Rehabilitation
Chen YM, et al. Yoga Phys Ther Rehabil. 6: 1084.
www.doi.org/10.29011/2577-0756.001084
www.gavinpublishers.com

OPEN ACCESS

GAVIN PUBLISHERS

Research Article

Exploration of Gene Expression Characters of Mouse M-1 Cells Treated by Qigong

Yu-ming Chen[1], Yan-peng Zhao[1], Lin Wang[1], Qian Feng[1], Jie Teng[1], Yu Chen[1], Meng-mei Li[1], Zhong-zhen Cai[1], Cai-xia Li[1], Chao Zhang[1], Glen Rein[2], Bing Wang[1*], Xue-mei Bai[1*]

[1]ENNOVA Institute of Life Science and Technology, ENN group, Langfang, Hebei, P.R. China

[2]Quantum Biology Research Lab, Ridgway, Colorado, USA

[*]**Corresponding authors**: Xuemei Bai, ENNOVA Institute of Life Science and Technology, ENN group; Langfang, Hebei, P.R. China.

Bing Wang, ENNOVA Institute of Life Science and Technology, ENN group; Langfang, Hebei, P.R. China.

Citation: Chen YM, Zhao YP, Wang L, Feng Q, Teng J, et al. (2021) Exploration of Gene Expression Characters of Mouse M-1 Cells Treated by Qigong. Yoga Phys Ther Rehabil. 6: 1084. DOI: 10.29011/2577-0756.001084

Received Date: 24 December, 2021; **Accepted Date:** 27 December, 2021; **Published Date:** 30 December, 2021

Abstract

Qigong is a traditional method of exercise in China and is effective in maintaining physical and mental health, and even in curing diseases. There are many reports about the beneficial effects of Qigong while the underlying functional mechanism has not been systematically explored at the molecular and cellular levels. In this study, we examined the effect of Qigong on gene expressions of mouse M-1 cells using the quantitative real-time polymerase chain reaction (qRT-PCR) and the transcriptome sequencing technology. Analysis result showed that several genes were upregulated the expression level related to cell proliferation and apoptosis by Qigong treatment. Enrichment analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) of differentially expressed genes (DEGs) showed that DEGs are related to many important signaling pathways in cells such as PI3K/AKT, and up-regulated DEGs are beneficial to cell growth, resistance to oxidative damage and apoptosis in vitro. Our study provided some important molecular insights into the positive effect of Qigong, which will help to comprehensively understand the biological mechanism of Qigong on human health and promote the application of Qigong treatment and the related research.

Keywords: Cellular Apoptosis; Qi; Qigong; Senescence; Transcriptome analysis

Introduction

In modern medical system, Qigong, Yoga, Taichi, and respiratory regulation are different methods of mind-body interaction, which are used in treatment of many human diseases [1]. Qigong, originated in ancient China, is one of the traditional methods for health care, health preservation and disease elimination [1-4]. Qigong utilizes the modulation of breath, physical activity and consciousness (breath, body and heart) as the means to achieve the purpose of maintaining health [1,5]. It is revealed that Qigong can alleviate chronic pain in adults and improve the health indexes in chronic heart patients [6,7].

There are many studies on the mechanism of psychosomatic interaction in disease treatment. Some studies revealed that psychosomatic interaction affected the neuroendocrine system, and reduced the expression of inflammation related genes and the activity of NF-κB signaling pathway, which was beneficial to relieve chronic stress [8,9]. Some studies showed that Qigong enhanced the immunity of cancer patients, helping them fight against cancer [10]. It was reported that Qigong could improve fatigue symptoms,

enhance immunity, change cortisol level and improve quality of life of cancer patients [11,12].

Qigong is divided into two categories, namely the internal Qigong and external Qigong [6,13]. Internal Qigong focuses on the physical body, which is practiced through body movement, meditation and rhythmic breathing. External Qigong is performed by a trained Qigong practitioner who uses their hands to emit Qi as a flow of energy acting onto the patient's body to treat diseases and produce therapeutic effects [6,14]. Compared with a larger number of clinic experiments about Qigong, some in vitro laboratory experiments about Qi were also conducted, which may give more objective results for evaluation of the biological function of Qigong [15-18]. To analyze the potent cytotoxic effect of Qi towards SCLC cell line, a global microarray analysis was performed, and 39 genes whose expression was altered by Qi treatment were identified [18]. Further semi-quantitative RT-PCR and real-time qPCR analyses confirmed the related gene expression levels of the proteins and enzymes. It was demonstrated that Qi induced apoptosis in cancer cells while protecting neurons from oxidative stress induced cell death via modulating signaling pathways and gene expression. In a recent study, Yan, et al. found Qi induced apoptosis in A549 cells, resulting in a pronounced reduction in viability and clonogenic formation [17]. The process was associated with inhibition of phosphorylation of AKT and ERK1/2, and reduced expression of anti-apoptotic proteins BCL-XL, XIAP and surviving. At present, researches have provided evidence for the effects of Qigong, and proved that differential gene expression played essential and principal roles in Qigong treatment. However, the functional mechanisms of these effects at the molecular level remained unclear due to the limitations of research technologies.

The effect of Qigong on different cells may involve a variety of signaling pathways and related vital genes, but until now, the relevant reports are still very limited. In yeast, the function of the NAD-dependent protein deacetylase silent information regulator 2 (Sir2) in mediating lifespan extension was thoroughly studied [19,20]. And in mammals, Sirt1 gene was the most closely related homologue of yeast Sir2 and involved in a wide variety of cellular processes from ageing to cancer [21,22]. Heat shock transcription factor 1 (Hsf1) was one of the four mammalian HSF family members and involved in the modulation of the lifespan-enhancing pathways [23,24]. Heat shock protein 32 (Hsp32), also known as heme oxygenase 1(HO-1), plays against the oxidative stress damage and attenuates the subsequent cell senescence [25,26]. Sirt1, Hsf1 and Hsp32 are all vitagenes that are involved in preserving cellular homeostasis during stressful conditions [27,28]. Researches have proved that activation of vitagenes can differentially modulate cellular activities inducing apoptosis/cell death in abnormal cancer cells but protecting against carcinogenesis and neurodegeneration in normal cells [29].

Transcriptome analysis is a widely used technology in animals, plants and microorganisms to study their gene expression and variation at the molecular level [30-32]. In a report, the biological effects of electromagnetic radiation emitted by the "SG III" prototype laser facility on HaCat cells and PC12 cells were studied via transcriptome analysis, and it was revealed that the damage of cells was dose-dependent, and the most significantly differentially expressed genes involved in proliferation, transformation, necrosis, etc. [33]. In a study, it was shown that the electromagnetic field treatment in the myometrium affected the expression of genes involved in defense and immune responses [34]. In another study, through RNA-seq analysis, RANKL-induced RAW264.7 cells under high static magnetic field were analyzed. As the result, a total number of 197 DEGs were discovered and the related molecular mechanism was discussed [35]. Therefore, transcriptome analysis was a very sensitive assay method for weak treatment such as electromagnetic radiation, magnetic field, Qigong, etc. Through the application of transcriptome analysis, we were able to distinguish the miniscule effects induced by a normal experienced Qigong practitioner, so as to systematically analyze Qigong on large scale to reveal its essence and working mechanism.

In this study, we invited experienced qigong masters to treat cells, detect multiple genes related to immune and aging, and perform transcriptome sequencing to provide more evidence and vision for comprehensive understanding of the biological function of Qigong and Qi.

Method

Cells and treatment

Mouse M-1 renal collecting duct cell line was obtained from National Infrastructure (Beijing, China, http://www.cellresource.cn). M-1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 medium with 10% Fetal Bovine Serum (FBS) and placed at 37 ˚C with 5% CO_2 incubator. A randomized dual-blind design was used to examine the effect of Qi on cultured cells as described by Yan, et al. [18]. The Qi provider named Guowei Lin, an experienced Qigong practitioner, was invited to the laboratory to treat the cells and not involved in any cell collection or assay process. The M-1 cells were seeded at a density of $5\sim6\times10^5$ cells/plate into 10 cm plates. After 24 hours of culture, the cells were randomly divided into two groups: Qi treatment and non-treatment, each group has 9 culture dishes. The cell dishes of the Qi treatment group (QT) were placed on a table at room temperature (RT). The Qi was applied from Qigong provider's fingers to plates, followed by Qi treatment for 15 min and the distance between the hands and plates was about 5 cm [14,18], Which means that there is no any physical contact at all. At the same time, control group (QC) were placed on the desk in another room without any treatment. After that, immediately put all the cells back into

the same incubator, and incubate them for additional 4, 24 and 48 h, respectively. Cell was collected from dish, froze in liquid nitrogen, and stored at -80 ˚C refrigerator for further analysis. The sample remains labeled in the code until the sample measurement is completed.

RNA isolation and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total cellular RNA was extracted using the TaKaRa MiniBEST Universal RNA Extraction Kit (TaKaRa, 9767) according to the manufacturer's instructions after Qi treatment. The quantity of RNA was assessed by NanoPhotometer (Implen, Germany). RNA was then reverse transcribed to cDNA utilizing Reverse Transcriptase M-MLV (TaKaRa, 2641A) according to the manufacturer's instructions. Gene expression was quantified by qRT-PCR using TB Green® Premix Ex Taq™ II (TaKaRa, RR820) and a quantitative real time-PCR system (ABI, Quant Studio 3). The primers specific for Sirt1, Hsf1, Hsp32, p16, Tert, and β-actin were listed in Table 1. The relative abundance of each target was obtained by normalization to β-actin using $2^{-\Delta\Delta CT}$ method.

Name	Sequence (5'→3')	Accession
mβ-actin -F	agatcaagatcattgctcctcct	NM_007393.5
mβ-actin -R	acgcagctcagtaacagtcc	
mSirt1 -F	gatacggagaggcccgaatg	AY377984.1
mSirt1 -R	agttcccaatgctggtggag	
mHsf1 -F	catgagcctgcctgacctg	NM_001331152.1
mHsf1 -R	caccagctgctttcctgagt	
mHsp32 -F	gcctccagagtttccgcata	NM_010442.2
mHsp32 -R	aggaagccatcaccagcttaaa	
mP16 -F	ttcttggtgaagttcgtgcg	BC058190.1
mP16 -R	cgtgaacgttgcccatcatc	
mTert -F	acggatatctggccctctgt	AF051911.1
mTert -R	atttgcctgatggggttggt	

Table 1: Primers used in this study.

cDNA Library Construction and RNA Sequencing

Cellular total RNA was sent to Sangon Biotech (Shanghai) Co., Ltd for transcriptome analysis. Sequences were analyzed using Illumina HiSeqTM system (Sangon Biotech, Shanghai, China). The quality of raw data was further assessed and analyzed by FastQC software. Nucleic acid sequences containing low quality base or unknown base were excluded. The remaining sequences from the six samples were compared with the reference transcriptome of Mus musculus by HISAT2 software. Expression abundance of genes in each sample was calculated in terms of transcripts per million (TPM) by StringTie software. Data was obtained from 3 independent biological replicates.

Identification of Differentially Expressed Genes (DEGs)

The DEGs between QT and QC groups were analyzed using DEGseq software, and genes with P value < 0.01 and |log2 (fold change) |>0.8 were considered significantly differentially expressed. The energy information of Qigong has less effect on cell growth or / and cell function than substances, so the filter conditions for DEGs are also reduced. Fold change meant the magnification of expression levels of the specific genes in samples of the QT group compared with QC group.

GO and KEGG Enrichment Analysis of DEGs

The Gene Ontology (GO), subordinate cellular component (CC), molecular function (MF) and biological process (BP), enrichment analysis of DEGs were analyzed using topGO software. Significantly, enriched GO terms were thereby obtained. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used for functional annotation of the DEGs using clusterProfiler software. P value < 0.05 is considered significant.

KEGG Pathway Network

Draw multiple DEGs-related KEGG signaling pathways into a network, and construct the relationship between the signaling pathways and their regulatory genes. It involves protein-protein interaction, protein-metabolic pathways interaction, and ultimately points to potential biological functions.

Results

Qi Affected the Expression of Several Senescence-Related Genes of M-1 Cells

In the experiment, we found that some of the mitochondrial-related biochemical indicators of the cell changed after Qi treatment, including increase in mitochondrial membrane potential and total adenosine phosphate, decrease in reactive oxygen species (not shown), then the expression levels of senescence- or apoptosis-related genes were examined by qRT-PCR. Results showed that the average expression levels of genes *Sirt1, Hsf1*, *Hsp32* and *P16* were upregulated from three independent experiments after Qi treatment for 4h (Figure 1). Since these genes have beneficial effect in prolonging the lifespan or anti-apoptosis, it was clear that Qi played a positive role in maintaining cells in good growth status.

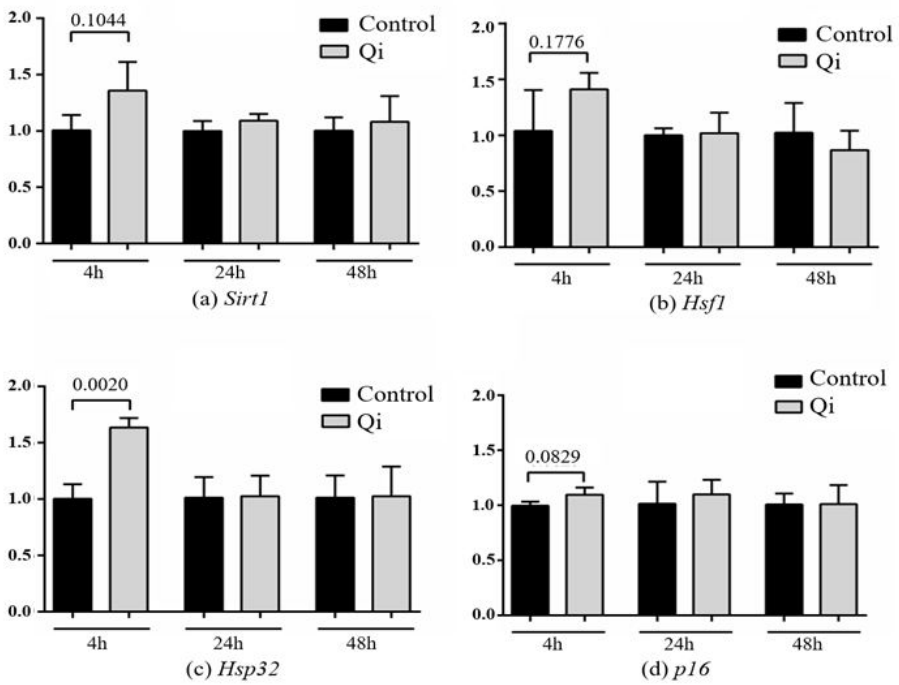

Figure 1: Effects of Qi on the transcription levels of senescence-related genes. M-1 cells were treated by Qi for 10 min. Total cellular RNA was isolated at 4, 24 and 48 hours post Qi treatment and reverse transcribed to cDNA, respectively. Levels of *Sirt1* (A), *Hsf1* (B), *Hsp32* (C) and *P16* (D) genes were detected by qRT-PCR, respectively. The number on the top of the columns represent the P value. Error bar represent the standard deviation of the mean of three independent experiments.

Identification of DEGs between the QT and QC groups

In this work, the abundance of genes in the samples was calculated in terms of transcripts per million (TPM). To verify the transcriptome data, the genes *Tert, Sirt1, Hsf1, Hsp32* and *P16* were compared with that of RT-qPCR results (Figure 2). As expected, the changes in gene expression of the selected genes obtained from RT-qPCR was similar in magnitude to deep sequencing results, indicating that the results from RNA-Seq were reliable, and subsequent analysis can be performed. Gene in cells with |log2(fold change) |>0.8 and P value<0.01 between the two groups were considered as DEGs. And the result showed that there were 48 DEGs between the QT and QC groups. This result indicated the alterations in gene expression after Qi treatments for 10 min.

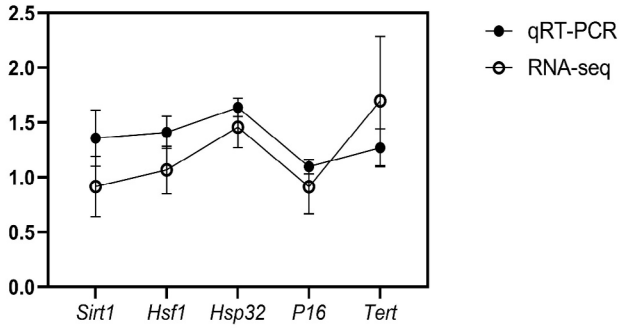

Figure 2: Trends comparison of two methods for relative gene expression. the relative expression levels are calculated and compared the trends between RT-qPCR and RNA-Seq methods.

Gene Symbol	TPM					
	QC1	QC2	QC3	QT1	QT2	QT3
Sirt1	11.35856	11.57755	12.61705	12.60767	12.87882	7.061343
Hsp32	54.63339	55.9141	62.02529	55.41522	59.91782	53.89552
P16	523.6194	447.0647	459.8406	475.3524	414.1294	741.8709
Hsf1	60.4941	66.18647	70.14391	64.74683	59.12419	86.15067
Tert	1.474731	1.651103	1.564749	2.223311	2.02626	3.711579

Table 2: Sequencing TPM of experimental detection gene.

Function Enrichment Analysis of DEGs

GO analysis is divided into three categories: MF, CC, and BP, which describe the molecular functions that gene products may perform, the cellular environment in which they are located, and the biological processes involved. GO functions with P value less than 0.05 considered to be significantly affected. According to the functional enrichment results, 236 BP and 82 MF terms were statistically significantly enriched in DEGs, and the top 20 terms are enriched candidate gene number more than one **(**Figure 3a-c**)**. The most enriched BP terms include negative regulation of apoptotic process, cellular response to insulin stimulus and positive regulation of protein ubiquitination. Among them, the process of negative regulation of apoptotic process was significantly activated, with six DEGs involved, including *Hpn, Apc, Arrb1, Xiap, Hspa5, Nupr1*. endoplasmic reticulum membrane the most enriched CC term. The most enriched MF terms were ubiquitin protein ligase binding, ion channel binding and insulin-like growth factor receptor binding.

KEGG database was used to analyze KEGG pathway classification of DEGs between the QT and QC group, with P value less than 0.05 considered significantly affected pathways. The results showed that eleven KEGG pathways were statistically significantly enriched in DEGs by Qi, and a total of 11 genes were mapped into signal pathways (Figure 3d), Among them, the insulin resistance pathway (KO 04931) and AMPK signaling pathway (KO 04152) and FoxO signaling pathway (KO 04068) were significantly enriched by genes Herpud1, Insr, Pck2, Trib3, Gpt2 et al. (Table 3).

ID	Description	GENE	P value
KO 04931	Insulin resistance	Insr; Trib3; Pck2	0.0021
KO 00130	Ubiquinone and other terpenoid-quinone biosynthesis	Vkorc1	0.0259
KO 00220	Arginine biosynthesis	Gpt2	0.0444
KO 00603	Glycosphingolipid biosynthesis – globo and isoglobo series	Fut1	0.0375
KO 00670	One carbon pool by folate	Aldh1l2	0.0444
KO 01210	2-Oxocarboxylic acid metabolism	Gpt2	0.0444
KO 04068	FoxO signaling pathway	Insr;Pck2	0.0382

KO 04152	AMPK signaling pathway	Insr;Pck2	0.0346
KO 04910	Insulin signaling pathway	Insr;Pck2	0.0415
KO 04928	Parathyroid hormone synthesis,secretion and action	Arrb1;Pde4d	0.0263
KO 05032	Morphine addiction	Arrb1;Pde4d	0.0195
KO 00020	Citrate cycle (TCA cycle)	Pck2	0.0736
KO 00250	Alanine, aspartate and glutamate metabolism	Gpt2	0.0847
KO 00601	Glycosphingolipid biosynthesis – lacto and neolacto series	Fut1	0.0625
KO 00620	Pyruvate metabolism	Pck2	0.0869
KO 03060	Protein export	Hspa5	0.0625
KO 04141	Protein processing in endoplasmic reticulum	Hspa5;Herpud1	0.0561
KO 04215	Apoptosis - multiple species	Xiap	0.0736
KO 04960	Aldosterone-regulated sodium reabsorption	Insr	0.0869
KO 04964	Proximal tubule bicarbonate reclamation	Pck2	0.0512

Table 3. The TOP20 KEGG pathways affected by Qi.

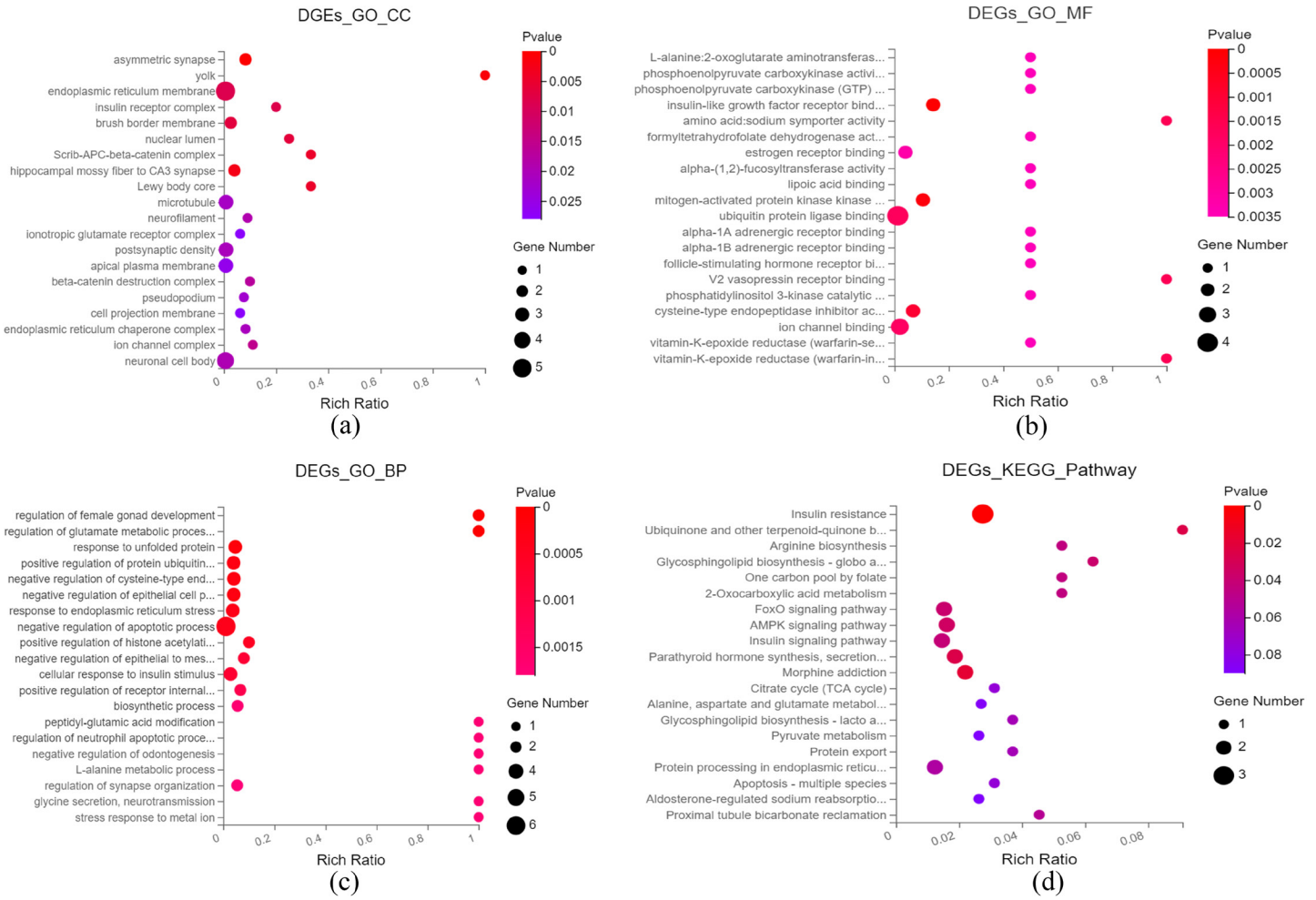

Figure 3: Scatter plot of top 20 enriched GO terms and KEGG pathways. Enrichment GO analysis of the cellular component (CC) (a), molecular function (MF) (b) and biological process (BP) (c), and enrichment KEGG pathway analysis (d). The circle size represents the number of enriched genes, and the color represents significance.

KEGG Pathway Network of the DEGs and Functions

The network of the top20 KEGG pathway and their corresponding DEGs indicated that the eight genes were the major force which drive the subjective cells changed their cellular functions after Qi treatment. Network map can be divided into two parts, one is the carbon cycle related to intracellular glucose with Pck2 gene as the core, including three metabolic cycles of one-carbon cycle, tricarboxylic acid cycle, and urea cycle, and three DEGs of Aldh1l2, Gpt2 and Pck2. The other is the response related to the environment change of cells with AKT as the core, involving cell apoptosis, oxidative stress, ATP production, cell growth and other cell activities, including Insr, Trib3, Arrb1 and other DEGs (Figure 4). The results were consistent with the previous reported that the Qi affected the apoptosis related biological processes in A549 cells [16] and in SCLC cell line NCI-H82 [15].

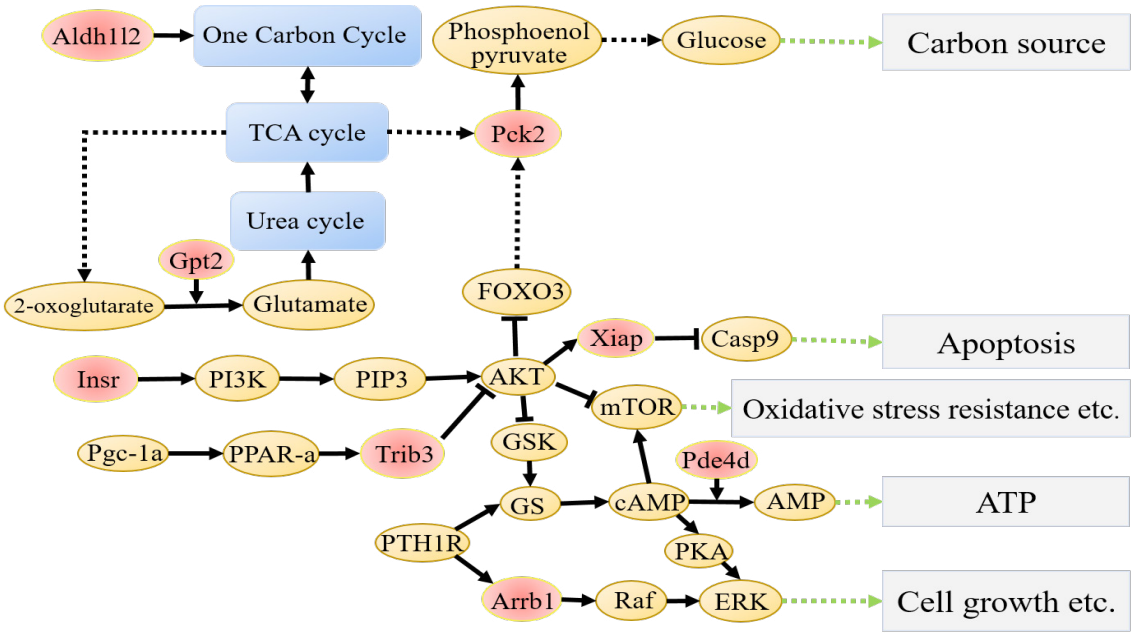

Figure 4: Interaction network of top 20 KEGG pathway and the connected DEGs. The network contained 5 cell process and 8 DEGs. The circles represent the gene and/or protein, among them, the reddish ones represent DEGs. The squares represent the functions, involving cell function and KEGG pathway.

Discussion

As a type of exercise which plays a positive role in promoting the mind-body interaction, Qigong has been used in the treatment and rehabilitation of various diseases for a long time. Based on statistical methods, the therapeutic effects were proved of Qigong on cancer, cardiovascular disease, chronic pain, hypertension, arthritis and stroke [3,10,36]. Despite the important function of Qigong in promoting health, few studies were conducted to explore the underlying mechanisms with development of scientific technologies to investigate the molecular mechanism of Qigong, mainly focusing on the effect of Qi on various cells [14,17,37].

Some studies have shown the influence of Qigong on gene expression in different cells. Yan et al. found that Qi could cause the death of small cell lung cancer cells by regulating biological processes such as proliferation, migration and sugar metabolism [15]. Studies in colorectal cancer cells showed that Qi caused cell cycle arrested and apoptosis by inhibiting the activation of Akt, extracellular regulated protein kinases 1/2 (Erk1/2) and nuclear factor kappa-B (NF-κB) [37]. In addition, Qi can also induce the apoptosis of prostate cancer cells and breast cancer cells [38,39]. Via evaluating changes in the expression of genes associated with cellular stress response in peripheral mononuclear blood cells in healthy women, significant differential expression in NF-kB, CCL2, IL10, HSF1 and HSPA1A were detected after 4 days of Qigong training [40].

In our study, the DEGs expression level in figure 4 were all upregulated by the transient treatment of Qi. Using the transcriptome analysis, several genes in Qi-treated cells were revealed to be significantly affected involving in glucose metabolism and AKT pathway. Oxidative phosphorylation-independent mitochondrial GTP dependent phosphoenolpyruvate cycle couple phosphoenolpyruvate carboxykinase 2 (Pck2), which catalyzes the first rate-limiting enzyme in the liver gluconeogenesis pathway, to pyruvate kinas in the liver and pancreatic islets, amplifies glucose-stimulated insulin secretion and improves insulin sensitivity in the body to regulate glucose homeostasis. It is important for nutrient-stimulated insulin secretion and can provide targeted therapeutic opportunities. In diabetic animal models, it showed decreased gluconeogenesis, increased red blood cell glycolysis, and decreased liver steatosis [41,42]. Mitochondrial glutamate-pyruvate transaminase 2 (GPT2) expression contributes to cell survival and growth by sustaining the Tricarboxylic Acid (TCA) cycle anaplerosis via activating transcription factor 4 after the inhibition of glutaminase [43]. Mitochondrial folate enzyme Aldh1l2, a member of aldehyde dehydrogenases family, converts 10-formyltetrahydrofolate to CO_2 and tetrahydrofolate, which is an important molecular for one carbon cycle. Coenzyme A, plays an important factor for regulating sugar; fat and protein metabolism, has a strong deficiency impact on liver metabolism upon the Aldh1l2 loss. Specifically, the decrease accumulation of methylmalonic acid, acylcarnitines, intermediates of TCA cycle and ATP level in the liver are in agree-

ment with a low abundance of CoA [44]. Glucose is the carbon source for cell metabolism. Up-regulation of three DEGs is conducive to the production of glucose. From this, we can infer that Qi treatment can promote glucose synthesis in cells and provide cells with more energy.

PI3K/AKT/mTOR, MAPK/ERK, and SIRT1/FOXO which are signaling pathways for regulating endocrine response, necrotic, stress resistance, autophagy, and apoptosis and cell growth and differentiation. Insulin is a major endocrine hormone that activates the intracellular signal cascade through insulin receptor (INSR) and Insulin Receptor Substrate (IRS) proteins. In adipocytes/tissues, the production of leptin is stimulated through PI3K/PDE3B-dependent mechanisms, and through PI3K/AKT and the activation transcription factors SREBP1, C/EBP-α and Sp1. In endothelial cells, insulin increases endothelial cell migration and enhances the formation of new blood vessels by activating the PI3K/AKT pathway. In addition, ERK can also be activated by INSR-A [45]. Multifunctional-endoplasmic reticulum stress induces protein TRIB3 to ubiquitinate and degrade a variety of proteins, and negatively regulate multiple signaling pathways. In the PI3K/AKT/mTOR pathway, TRIB3-mediated inhibition of AKT phosphorylation reduces insulin signaling and cell survival [46]. As an adaptor protein, β-arrestin-1(Arrb1) can regulate G protein-coupled receptor (GPCRs)-mediated glucagon-like peptide-1 activation of ERK1/2 signals to regulate insulin secretion and cell survival. It can also activate AKT/PKB and other GPCR signaling pathways [47]. phosphodiesterase-4D (Pde4d) can regulate the signal transduction of cAMP-PKA-SIRT1-Akt-Bcl-2/Bax pathway in nerve cells [48]. X-linked inhibitor of apoptosis (Xiap), which is E3 ubiquitin-protein ligase, inhibited the apoptosis process through modulating the levels of B-cell lymphoma-2 and delaying the release of cytochrome c and apoptotic protease activating factor 1 from mitochondria, thus inhibiting the apoptosis of renal cell carcinoma [49]. The results show that Qi is closely related to the biological processes of AKT, ERK and other signaling pathways in cells.

It was important to note that Qi appeared to have opposite effects on the apoptosis of cancer and normal cells [17,18]. Qi was proved to upregulate the activity of normal cells of fibroblasts and human umbilical vein endothelial cells by the modulation of specific genes [17]. In consistent with the report, our present study showed that Qi upregulated the transcription levels of several anti-senescence and anti-apoptosis genes in M-1 cells (Figure 2 and Table 3). In this study, the gene Xiap was upregulated in M-1 cells (normal cells) by Qi treatment (Table 3), while its expression was reduced in human lung adenocarcinoma A549 cells (cancer cells), according to the report by Yan, et al. [16]. Considering the remarkable and opposite role of Qi in cancer and normal cells, it was really a very safe and beneficial medical treatment.

Our experiments also showed that even the Qi was from a normal experienced Qigong practitioner (not a famous Qigong Master), it also caused similar biological effects on M1 cells. This shows that Qi may not be born with it, but it can be obtained through practice. Although the effect on gene expression in cells is not great, it is of great significance to promote the application of qigong therapy and research. Since the normal experienced Qigong practitioner also had the ability to conducted Qi treatment effectively, further large-scale studies about Qi treatment with multiple Qigong practitioners in different kinds of cells or patients were expected so as to reveal the underlying functional mechanisms.

Conclusion

In general, integrative medicine approaches have gained significant interest in recent years to provide a solution for the health care challenges. Qigong is one of the most widely used integrative medicine approaches. Our study preliminarily reveals an important role of Qi in regulation of cell senescence and apoptosis. After the short time treatment, the Qi persuasively caused some obvious effects on the M-1 cells, especially at the molecular level, DEGs can indirectly regulate cell process through the AKT pathway, such as cell growth, metabolism, anti-oxidation, anti-apoptosis, etc. The results of this work are of great significance for comprehensively understanding the biological mechanism of qigong and the popularization of qigong exercises.

Acknowledgements

The authors would like to thank the ENN Group for funding this research. Thank the team members for their hard work and dedication to have this manuscript. We are also grateful to the editor and anonymous reviewers for their constructive criticisms and suggestions, which significantly improved this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

1. Buric I, Farias M, Jong J, Mee C (2017) What Is the Molecular Signature of Mind-Body Interventions? A Systematic Review of Gene Expression Changes Induced by Meditation and Related Practices. Front. Immunol 8: 670.
2. Lee MS, Pittler MH, Ernst E (2007) Tai chi for rheumatoid arthritis: systematic review. Rheumatology (Ox-ford) 46: 1648-1651.
3. Marks R. (2017) Qigong Exercise and Arthritis. Medicines (Basel): 4.
4. Tao WW, Jiang H, Tao XM, Jiang P (2016) Effects of Acupuncture, Tuina, Tai Chi, Qigong, and Traditional Chinese Medicine Five-Element Music Therapy on Symptom Management and Quality of Life for Cancer Patients: A Meta-Analysis. J Pain Symptom Manage 51: 728-747.
5. Jiménez-Martín PJ, Liu H (2018) Exploring the health advantages and disadvantages of static and dynamic postures of Qigong and its use as a Traditional and Complementary Medicine. European Journal of Integrative Medicine 24: 61-64.

6. Bai Z, Guan Z, Fan Y, Liu C (2015) The Effects of Qigong for Adults with Chronic Pain: Systematic Re-view and Meta-Analysis. Am J Chin Med 43: 1525-1539.

7. Chan CL, Wang CW, Ho RT, Ho AH (2012) A systematic review of the effectiveness of qigong exercise in cardiac rehabilitation. Am J Chin Med 40: 255-267.

8. Bower JE, Irwin MR (2016) Mind-body therapies and control of inflammatory biology: A descriptive re-view. Brain Behav Immun 51: 1-11.

9. Mulero MC, Huxford T , Ghosh G (2019) NF-kappaB, IkappaB, and IKK: Integral Components of Im-mune System Signaling. Adv Exp Med Biol 1172: 207-226.

10. Chan CL, Wang CW, Ho RT, Ng SM (2012) A systematic review of the effectiveness of qigong exercise in supportive cancer care. Support Care Cancer 20: 1121-1133.

11. Klein PJ, Schneider R, Rhoads CJ (2016) Qigong in cancer care: a systematic review and construct analysis of effective Qigong therapy. Support Care Cancer 24: 3209-3222.

12. Zeng Y, Luo T, Xie H, Huang M (2014) Health benefits of qigong or tai chi for cancer patients: a system-atic review and meta-analyses. Complement Ther Med 22: 173-186.

13. Gouw VXH, Jiang Y, Seah B, He H (2019) Effectiveness of internal Qigong on quality of life, depressive symptoms and self-efficacy among community-dwelling older adults with chronic disease: A systematic review and meta-analysis. Int J Nurs Stud 99: 103378.

14. Chien CH, Tsuei JJ, Lee SC, Huang YC (1991) Effect of emitted bioenergy on biochemical functions of cells. Am J Chin Med 19: 285-292.

15. Yan X, Li F, Dozmorov I, Frank MB (2012) External Qi of Yan Xin Qigong induces cell death and gene expression alterations promoting apoptosis and inhibiting proliferation, migration and glucose metabolism in small-cell lung cancer cells. Mol Cell Biochem 363: 245-255.

16. Yan X, Shen H, Jiang H, Hu D (2018) YXQ-EQ Induces Apoptosis and Inhibits Signaling Pathways Im-portant for Metastasis in Non-Small Cell Lung Carcinoma Cells. Cell Physiol Biochem 49: 911-919.

17. Yan X, Shen H, Jiang H, Zhang C (2006) External Qi of Yan Xin Qigong differentially regulates the Akt and extracellular signal-regulated kinase pathways and is cytotoxic to cancer cells but not to normal cells. Int J Biochem Cell Biol 38: 2102-2113.

18. Yan X, Shen H, Zaharia M, Wang J (2004) Involvement of phosphatidylinositol 3-kinase and insulin-like growth factor-I in YXLST-mediated neuroprotection. Brain Res 1006: 198-206.

19. Kaeberlein M, McVey M, Guarente L (1999) The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes Dev 13: 2570-2580.

20. Tissenbaum HA, Guarente L (2001) Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. Nature 410: 227-230.

21. Huang J, Gan Q, Han L, Li J (2008) SIRT1 overexpression antagonizes cellular senescence with activated ERK/S6k1 signaling in human diploid fibroblasts. Plos One 3: e1710.

22. Wang Y, Liang Y, Vanhoutte PM (2011) SIRT1 and AMPK in regulating mammalian senescence: a critical review and a working model. FEBS Lett 585: 986-994.

23. Hsu AL, Murphy CT, Kenyon C (2003) Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science 300: 1142-1145.

24. Morley JF, Morimoto RI (2004) Regulation of longevity in Caenorhabditis elegans by heat shock factor and molecular chaperones. Mol Biol Cell 15: 657-664.

25. Calabrese V, Colombrita C, Sultana R, Scapagnini G (2006) Redox modulation of heat shock protein expression by acetylcarnitine in aging brain: relationship to antioxidant status and mitochondrial function. Antioxid Redox Signal 8: 404-416.

26. Even B, Fayad-Kobeissi S, Gagliolo JM, Motterlini R (2018) Heme oxygenase-1 induction attenuates senescence in chronic obstructive pulmonary disease lung fibroblasts by protecting against mitochondria dysfunction. Aging Cell 17: e12837.

27. Calabrese V, Cornelius C, Dinkova-Kostova AT, Iavicoli I (2012) Cellular stress responses, hormetic phytochemicals and vitagenes in aging and longevity. Biochim Biophys Acta 1822: 753-783.

28. Cornelius C, Perrotta R, Graziano A, Calabrese EJ (2013) Stress responses, vitagenes and hormesis as critical determinants in aging and longevity: Mitochondria as a “chi”. Immun Ageing 10: 15.

29. Calabrese V, Cornelius C, Dinkova-Kostova AT, Calabrese EJ (2009) Vitagenes, cellular stress re-sponse, and acetylcarnitine: relevance to hormesis. Biofactors 35: 146-160.

30. Huang L, Li J, Anboukaria H, Luo Z (2016) Comparative transcriptome analyses of seven anurans reveal functions and adaptations of amphibian skin. Scientific reports 6: 24069.

31. Li JX, He JJ, Elsheikha HM, Chen D (2019) Toxoplasma gondii ROP17 inhibits the innate immune re-sponse of HEK293T cells to promote its survival. Parasitol Res 118: 783-792.

32. Yu Y, Zhu D, Ma C, Cao H (2016) Transcriptome analysis reveals key differentially expressed genes in-volved in wheat grain development. The Crop Journal 4: 92-106.

33. Wei J, Shi Q, Xiong L, Xin G (2021) Transcriptome profiling of cells exposed to particular and intense electromagnetic radiation emitted by the “SG-III” prototype laser facility. Scientific Reports 11: 2017.

34. Drzewiecka EM, Kozlowska W, Paukszto L, Zmijewska A (2021) Effect of the Electromagnetic Field (EMF) Radiation on Transcriptomic Profile of Pig Myometrium during the Peri-Implantation Period-An In Vitro Study. Int J Mol Sci 22.

35. Huyan T, Peng H, Cai S, Li Q (2020) Transcriptome Analysis Reveals the Negative Effect of 16 T High Static Magnetic Field on Osteoclastogenesis of RAW264.7 Cells. Biomed Res Int 2020: 5762932.

36. Park JE, Kim JE, Jung S, Kim A (2017) The Effect of Dongeui Qigong for Prehypertension and Mild Es-sential Hypertension. Evid Based Complement Alternat Med 2017: 4274538.

37. Yan X, Shen H, Jiang H, Hu D (2013) External Qi of Yan Xin Qigong inhibits activation of Akt, Erk1/2 and NF-kB and induces cell cycle arrest and apoptosis in colorectal cancer cells. Cell Physiol Biochem 31: 113-122.

38. Yan X, Shen H, Jiang H, Hu D (2010) External Qi of Yan Xin Qigong Induces apoptosis and inhibits mi-gration and invasion of estrogen-independent breast cancer cells through suppression of Akt/NF-kB signal-ing. Cell Physiol Biochem 25: 263-270.

39. Yan X, Shen H, Jiang H, Zhang C (2008) External Qi of Yan Xin Qigong induces G2/M arrest and apop-tosis of androgen-independent prostate cancer cells by inhibiting Akt and NF-kappa B path-ways. Mol Cell Biochem 310: 227-234.

40. Polrola P, Wilk-Franczuk M, Wilczynski J, Nowak-Starz G (2018) Anti-inflammatory effect on genes ex-pression after four days of Qigong training in peripheral mononuclear blood cells in healthy women. Ann Agric Environ Med 25: 329-333.

41. Abulizi A, Cardone RL, Stark R, Lewandowski SL (2020) Multi-Tissue Acceleration of the Mitochondrial Phosphoenolpyruvate Cycle Improves Whole-Body Metabolic Health. Cell Metab 32: 751-766 e711.

42. Liu MX, Jin L, Sun SJ, Liu P (2018) Metabolic reprogramming by PCK1 promotes TCA cataplerosis, ox-idative stress and apoptosis in liver cancer cells and suppresses hepatocellular carcinoma. Oncogene 37: 1637-1653.

43. Kim M, Gwak J, Hwang S, Yang S (2019) Mitochondrial GPT2 plays a pivotal role in metabolic adapta-tion to the perturbation of mitochondrial glutamine metabolism. Oncogene 38: 4729-4738.

44. Krupenko NI, Sharma J, Pediaditakis P, Helke KL (2020) Aldh1l2 knockout mouse metabolomics links the loss of the mitochondrial folate enzyme to deregulation of a lipid metabolism observed in rare human disorder. Human Genomics 14: 41.

45. Cignarelli A, Genchi VA, Perrini S, Natalicchio A (2019) Insulin and Insulin Receptors in Adipose Tissue Development. Int J Mol Sci 20.

46. Mondal D, Mathur A, Chandra PK (2016) Tripping on TRIB3 at the junction of health, metabolic dys-function and cancer. Biochimie 124: 34-52.

47. Dalle S, Ravier MA, Bertrand G (2011) Emerging roles for beta-arrestin-1 in the control of the pancreatic beta-cell function and mass: new therapeutic strategies and consequences for drug screening. Cell Signal 23: 522-528.

48. Wang Y, Gao S, Zheng V, Chen L (2020) A Novel PDE4D Inhibitor BPN14770 Reverses Scopola-mine-Induced Cognitive Deficits via cAMP/SIRT1/Akt/Bcl-2 Pathway. Front. Cell Dev. Biol 8: 599389.

49. Chen C, Liu TS, Zhao SC, Yang WZ (2018) XIAP impairs mitochondrial function during apoptosis by regulating the Bcl-2 family in renal cell carcinoma. Exp Ther Med 15: 4587-4593.