

## Chapter

# Energy Metabolism Heterogeneity-Based Molecular Biomarkers for Ovarian Cancer

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## Abstract

Energy metabolism heterogeneity is a hallmark in ovarian cancer; namely, the Warburg and reverse Warburg effects coexist in ovarian cancer. Exploration of energy metabolism heterogeneity benefits the discovery of the effective biomarkers for ovarian cancers. The integrative analysis of transcriptomics (20,115 genes in 419 ovarian cancer samples), proteomics (205 differentially expressed proteins), and mitochondrial proteomics (1198 mitochondrial differentially expressed proteins) revealed (i) the upregulations of rate-limiting enzymes PKM2 in glycolysis, IDH2 in Krebs cycle, and UQCRH in oxidative phosphorylation (OXPHOS) pathways, (ii) the upregulation of PDHB that converts pyruvate from glycolysis into acetyl-CoA in Krebs cycle, and (iii) that miRNA (hsa-miR-186-5p) and RNA-binding protein (EIF4AIII) had target sites in those key proteins in energy metabolism pathways. Furthermore, lncRNA SNHG3 interacted with miRNA (hsa-miR-186-5p) and RNA-binding protein (EIF4AIII). Those results were confirmed in the ovarian cancer cell model and tissues. It clearly concluded that lncRNA SNHG3 regulates energy metabolism through miRNA (hsa-miR-186-5p) and RNA-binding protein (EIF4AIII) to regulate the key proteins in the energy metabolism pathways. SNHG3 inhibitor might interfere with the energy metabolism to treat ovarian cancers. These findings provide more accurate understanding of molecular mechanisms of ovarian cancers and discovery of effective energy-metabolism-heterogeneity therapeutic drug for ovarian cancers.

## Highlights

- Mitochondrial proteomics revealed the energy metabolism heterogeneity in ovarian cancers.
- LncRNA SNHG3 was related to ovarian cancer survival and energy metabolism with ovarian cancer TCGA analysis.
- SNHG3 was related to energy metabolism by regulating miRNAs and EIF4AIII based on GSEA and Starbase analyses.
- MiRNAs and EIF4AIII regulate the glycolysis, Krebs cycle, and OXPHOS pathways by targeting PKM, PDHB, IDH2, and UQCRH.

**Keywords:** ovarian cancer, iTRAQ, mitochondrial proteomics, TCGA, energy metabolism, SNHG3

## **1. Introduction**

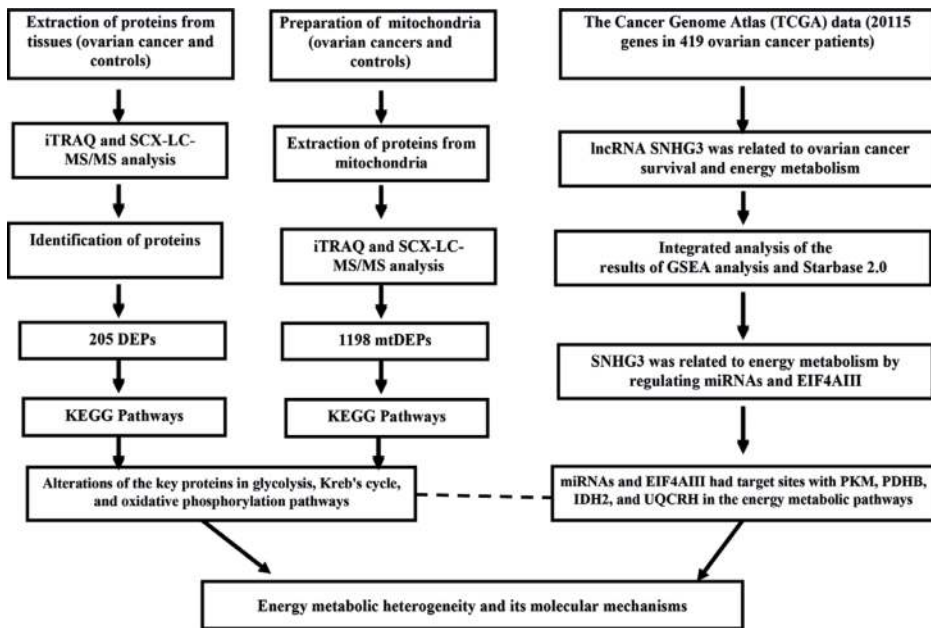
Ovarian cancer is a common gynecologic cancer with high mortality [1]. Despite chemotherapy, radiotherapy, surgery, and target therapy has previously been developed in ovarian cancers [2], the 5-year overall survival rate for patients who diagnosed with late stage III–IV disease is still very poor (about 30%). Because of the site of the ovaries and the certain clinical characteristics of epithelial cancers, it is a challenge to make early diagnosis [3]. Women with high-risk factors (e.g., family history, or BRCA mutations) plan for a follow-up visit with cancer antigen 125 (CA-125) monitoring and ultrasound, however, prospective validation of these physical examination or lab tests remain elusive [4]. The changes of energy metabolism are common in cancer cells, which might be potential biomarkers and therapeutics targets [5]. During the last decade, a great attention has been paid to metabolic reprogramming of cancer. However, cancer basic studies fail to reach a consistent conclusion on mitochondrial function in cancer energy metabolism [6]. The traditional view of Warburg was that cancer cells undergo aerobic glycolysis, which refers to the fermentation of glucose to lactate in the presence of oxygen as opposed to the complete oxidation of glucose, thus brought attention to the role of mitochondria in tumorigenesis [7]. A previous study found that the glycolysis enzyme PKM2 is important for cancer metabolism and tumor growth, which can improve activity and expression of PKM2 [8]. On the contrary, mitochondria were observed dysfunction, including the decreased effectiveness of Krebs cycle and electron transfer chain (ETC) complexes decoupling [9]. However, a novel ‘reverse Warburg effect’, was put forward in 2009 and impacted previous perceptions on cancer metabolism [10]. In this model of reverse Warburg chain, cancer cells and the cancer-associated fibroblasts (CAFs) become metabolically coupled. Interactions between cancer cells and tumor-microenvironment (TME) highly affect proliferation, energy metabolism, metastasis, and relapse of carcinoma [11]. Cancer cells secrete a large amount of ROS into microenvironment to enhance oxidative stress in CAFs. If the inflammatory reaction, autophagy, loss of stromal caveolin-1 (Cav-1), and nitric oxide synthase (NOS) are increased in CAFs, there is a good chance for progression of aerobic glycolysis [12]. Consequently, CAFs secrete plenty of energy-rich fuels to TME, including ketone bodies, lactate, pyruvate, and fatty acids. In turn, the nourishment ‘feed’ mitochondrial oxidative phosphorylation and ATP supplements [13]. In this process, mono-carboxylate transporters (MCTs) were highly expressed in both cancer cells and CAFs to be involved in some regulations. Immunohistochemistry result demonstrates that MCT4 was distributed specifically in CAFs in human breast cancers, which implicated in lactate efflux progress; while MCT1 participated in lactate uptake, and significantly upregulated specifically in kinds of cancer cells [14]. Thus evidence indicates limitations of ‘the Warburg effect’. However, some studies demonstrated that aerobic glycolysis was not the dominant energy metabolism approach for many human cancer cell lines. In the past decades, studies on Warburg and reverse Warburg effects in cancers have formed a new frontier regarding additional roles of mitochondria in a cancer, and multiple functions of mitochondria have been identified in tumorigenesis [15].

High-throughput proteomics approach provides a scientific evaluation of protein expression. Functional proteomics offers more subtle clues, due to a greater attention paid to subcellular proteome research [16]. However, the subcellular proteomics of ovarian cancer mitochondrial proteins has not been elucidated. Mitochondria are the center of energy metabolism in eukaryotic cells, and also involved in other functions, such as cell signaling, cellular differentiation, cell death, and maintaining control of the cell cycle and oxidative stress regulation [17]. Those mitochondria-mediated biological processes are so closely associated

with tumor relapse or metastasis. Thus, cancer therapeutics should urgently find a way to explore molecular mechanisms of mitochondrion during tumorigenesis and tumor progression [18]. Inside the cancer cell appeared structural and morphological alterations of the mitochondria, and variations of morphology and performance are presumably associated with mitochondrial differentially expressed proteins (mtDEPs) [19]. A slight increase in research on ovarian cancer has occurred in recent years, quantitative proteomic analysis of mitochondria from human ovarian cancer cells and their paclitaxel-resistant sublines proved that the chemoresistance mechanisms were partly related to the mitochondria [20]. Mitochondria similarly impart considerable flexibility for tumor cell growth and survival in otherwise harsh environments such as during nutrient depletion, hypoxia and cancer treatments, and are therefore key players in tumorigenesis [15]. The subcellular proteomics of ovarian cancer mitochondrial proteins may offer new insights into aspect of tumor development.

Regeneration of energy metabolism plays crucial roles in the pathogenesis and development of cancer since it accelerates cancer cell growth, cell cycle, proliferation and metastasis [21]. The impact of non-coding RNAs (ncRNAs) has profoundly touched the fields of human cancers, cell biology, functional genomics, and drug therapy. Long non-coding RNAs (lncRNAs) (>200 nucleotides) and microRNAs (20–24 nucleotides) have attracted much attention, which acted as key regulators in the cellular biological processes, gene expression, gene regulation, basic biological functions of eukaryotic genomes, and post-transcriptional regulation of mRNA [22]. Recent studies demonstrated that lncRNAs were widely used as biomarkers for the diagnosis and prognosis of malignant tumors [23], and some lncRNAs can even act as the new therapeutic targets [24]. More and more researchers have turned attention to the mechanism between non-coding RNAs and malignant tumors. lncRNAs affects on energy metabolism-related signaling pathways induced epigenetic regulation [25]. MicroRNAs can silence gene expression by binding to 3' untranslated region (3'UTR) sequences in their target messenger RNAs (mRNAs), resulting in the inhibition of translation or mRNA degradation, but the interaction of lncRNAs with microRNAs can hamper this effect [26]. The present results revealed that lncRNA FOXD2-AS1 acted as a tumor promoters partly through EphB3 inhibition by directly interacting with lysine (K)-specific demethylase 1A (LSD1) and zeste homolog 2 (EZH2), which indicates that lncRNA-target gene-carcinogenesis axis for cancers does exist [27]. Here, it emphasizes important scientific associations of lncRNAs with energy metabolism in cancer cells. Increasing evidence indicates that lncRNAs play significant roles in cancer metabolism, and explore the potential mechanisms that could help elucidate regulation axis or network and provide a new direction for clinical management of different malignant phenotypes [28]. In our previous research, iTRAQ-based quantitative proteomics identified 1198 mitochondrial differentially expressed proteins (mtDEPs) between mitochondria samples isolated from human ovarian cancer and control tissues [29] and 205 differentially expressed proteins (DEPs) between human ovarian cancers and controls tissues [39]. The TCGA database includes 20,115 genes in 419 ovarian cancer samples. The conjoint analysis of 1198 mtDEPs, 205 DEPs, and 20,115 TCGA data in ovarian cancers investigated the biological pathways and molecular mechanisms of SNHG3-downstream genes-energy metabolism axis. lncRNA SNHG3 was associated with survival for ovarian cancers, and further gene set enrichment analysis proved the roles of SNHG3 in the energy metabolism through miRNAs and RNA binding protein EIF4AIII to target genes, including PKM, PDHB, IDH2, and UQCRH [29].

**Figure 1** showed the experimental flow-chart of integrative analysis of 1198 mtDEPs [29], 205 DEPs [39], and 20,115 TCGA data in ovarian cancers [29] to reveal energy heterogeneity and its molecular mechanisms.



**Figure 1.**  
The experimental flow-chart to study energy metabolic heterogeneity and its molecular mechanisms.

## 2. Methods

### 2.1 Ovarian cancer mitochondrial DEP data and bioinformatic analysis

Mitochondria were separated from 7 ovarian cancer tissues (high-degrade, poorly or moderately differentiated carcinoma cells) (cancer group) and 11 control ovaries with benign gynecologic diseases (fibroids, adenomyosis, ovary serous cystadenoma, cervical intraepithelial neoplasia, atypical hyperplasia of endometrium, and pelvic organ prolapse) (control group), respectively [29]. The separated mitochondria were validated with electron microscopy and Western blotting. The extracted proteins from the prepared mitochondrial samples were used for iTRAQ-quantitative proteomics analysis. The extracted mitochondrial proteins from ovarian cancers and controls were analyzed with 6-plex iTRAQ labeling, SCX fraction, and LC-MS/MS. MS/MS data were used to determine proteins, and the intensities of iTRAQ reporter ions were used to determine each mitochondrial DEP. The mitochondrial DEPs were further analyzed by bioinformatics including GO functional enrichment and KEGG pathway enrichment with DAVID Bioinformatics Resources 6.7.

### 2.2 Ovarian cancer DEP data and bioinformatic analysis

Proteins were extracted from ovarian cancer and control tissues. The extracted proteins from ovarian cancers and controls were analyzed with 6-plex iTRAQ labeling, SCX fraction, and LC-MS/MS. MS/MS data were used to determine proteins, and the intensities of iTRAQ reporter ions were used to determine each mitochondrial DEP [39]. The mitochondrial DEPs were further analyzed by bioinformatics including GO functional enrichment and KEGG pathway enrichment with DAVID Bioinformatics Resources 6.7.

### 2.3 TCGA data of ovarian cancer patients and bioinformatic analysis

TCGA (<http://cancergenome.nih.gov/>) includes 20,115 genes of 419 ovarian cancer patients, in the level of transcriptome. Those genes were classified as coding/

non-coding RNAs (mRNAs/ncRNAs) provided by the GENCODE/ENSEMBL pipeline. lncRNA genes were considered as a type of genes that exclusively produce transcripts of the 'antisense'. The lncRNA survival analysis was performed by TANRIC ([http://ibl.mdanderson.org/tanric/\\_design/basic/index.html](http://ibl.mdanderson.org/tanric/_design/basic/index.html)). The Kaplan-Meier method was used to calculate overall survival. According to median value (3.39) of SNHG3 RNA expressions, 419 ovarian cancer patients were divided into SNHG3 high (>3.39; n = 210) vs. low (<3.39; n = 209) expression groups. TCGA data of two groups were analyzed with GSEA enrichment analysis. Moreover, the lncRNA expressions from Cancer Cell Line Encyclopedia (<https://portals.broadinstitute.org/ccle>), and chemosensitivity of tamoxifen from Genomics of Drug Sensitivity in Cancer (<http://www.cancerrxgene.org/>) were obtained for ovarian cancer cell lines. GraphPad Prism v6.0 (GraphPad Software, San Diego, CA, USA) was used to construct histogram.

#### **2.4 Integrative analysis of mitochondrial DEPs, tissue DEPs, and TCGA data with bioinformatics**

The integrated miRNA-lncRNA SNHG3, miRNA-target gene, RNA binding protein-lncRNA SNHG3, RNA binding protein-mRNA, and protein-protein signatures were identified. STRING 10.0 was used to predict interactions of chemicals and proteins. Chemicals were linked to other chemicals and proteins by evidence derived from experiments, databases and literature (<http://string-db.org/cgi/input.pl>). The large-scale CLIP-Seq data by starBase 2.0 (<http://starbase.sysu.edu.cn/mirCircRNA.php>) was used to construct SNHG3-miRNA, protein-miRNA, SNHG3-RNA binding protein, mRNA-RNA binding protein, and mRNA-microRNA-lncRNA interaction networks. The mitochondrial DEPs in ovarian cancers were input into STRING for protein-protein interaction analysis. Network visualizations were performed with Cytoscape 3.4.0 (<http://www.cytoscape.org/>). The binding sites of 3'UTR region of targeted genes were predicted with three publicly available databases (TargetScan, NCBI, and RNAhybrid), sequences of microRNA (>hsa-miR-186-5p MIMAT0000456 CAAAGAAUUCUCCUUUUGGGCU) and PDHB 3'UTR region. MicroRNA binding sites with PDHB were predicted with RNAhybrid database.

#### **2.5 Experimental validation in cell models**

Three ovarian cancer cell lines (TOV-21G, SK-OV3, and OVCAR-3), and one normal control cell line (IOSE80) from Keibai Academy of Science (Nanjing, China) were used. RPMI-1640 medium were used to culture TOV-21G and OVCAR-3 cells in 5% CO<sub>2</sub> atmosphere at 37°C. DMEM medium (Corning, NY, USA) were used to culture IOSE80 and SK-OV3 in 5% CO<sub>2</sub> atmosphere at 37°C, with supplementation of 10% fetal bovine serum (FBS, GIBCO, South America, NY, USA). (i) Transient transfection was performed with Lipofectamine 3000 reagents according to the manufacturer's instructions (Invitrogen, USA). SK-OV3, OVCAR-3, and TOV-21G were seeded in 6-well plates at 30–50% density. Cells were collected at 24–48 h after transfection, for next-step experiments. (ii) RNA extraction and quantitative real-time PCR (qRT-PCR) analyses. TRizol® Reagent (Invitrogen, CA, USA) was used to extract total RNAs. total RNAs were reversely transcribed into cDNAs and then used to perform qRT-PCR analysis to detect SNHG3 and its target genes, with β-actin as an internal control. (iii) 1D-SDS-PAGE and Western blotting was used to detect PKM, PFKM, PDHB, IDH2, CS, OGDHL, and UQCRH against the corresponding antibodies, with β-actin as internal control. (iv) Data were expressed as the mean ± SD of triplicates. Each experiment was repeated at least three times. In all cases, P < 0.05 was considered as statistical significance.

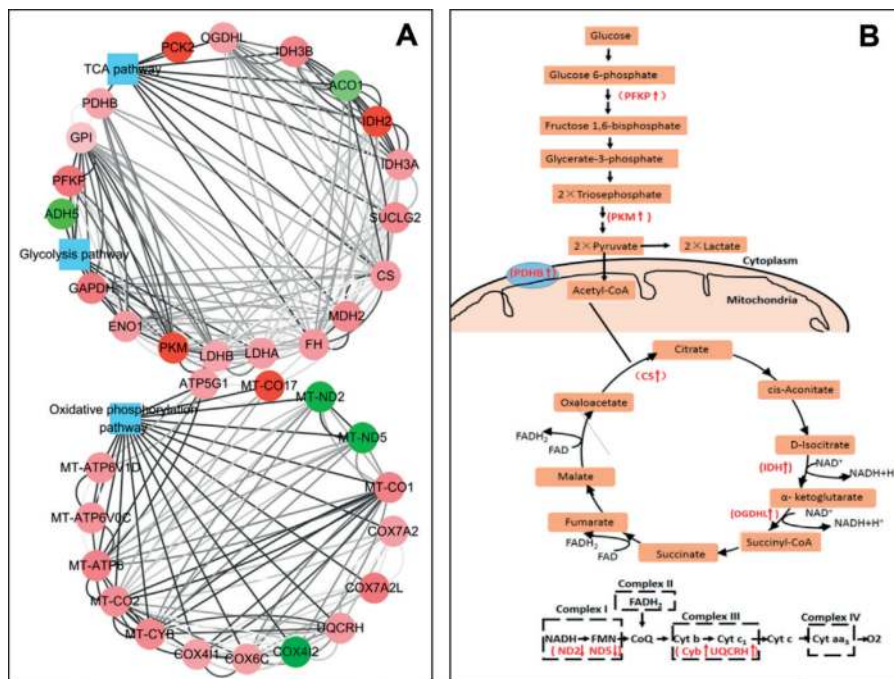
### 3. Results and discussion

#### 3.1 The changes of key proteins in the energy metabolism signaling pathways

The iTRAQ-based quantitative proteomics identified 1198 DEPs between mitochondria samples isolated from ovarian cancer and control tissues [29]. The statistically significant KEGG pathways were mined with DAVID Bioinformatics Resources from those mitochondrial DEPs between EOCs and controls, among which those DEPs were significantly enriched in the processes of Krebs cycle, and oxidative phosphorylation (OXPHOS) pathways. The key proteins (PDHB, IDH2, and UQCRH) were associated with aerobic oxidation to supply in the Krebs cycle, and oxidative phosphorylation was upregulated (Figure 2). Interestingly, those results were coincided with the reverse Warburg effect proposed in 2009 [10].

The iTRAQ-based quantitative proteomics identified 205 DEPs between ovarian cancer and control tissues [13], which revealed the upregulation of the key enzyme PKM2 in glycolysis pathway I in ovarian cancers. It was coincided with the Otto Warburg effect proposed in 1956 [30]. Warburg discovered that cancer cells tend to produce ATP by aerobic glycolysis, even though it is a less efficient pathway contrasted with OXPHOS. This popular system, called ‘Warburg effect’, has been the dominant mechanism of tumors for energy generations, while its relationship with tumorigenesis remains still unclear.

The research of the ‘Warburg effect’ mechanism of a cancer cell has never interrupted at home and abroad. PKM2, a splice isoform of the pyruvate kinase, serves as a major metabolic reprogramming regulator with an adjustable activity subjected to numerous allosteric effectors and post-translational modifications [31]. One observed that PKM2 modification was associated with an enhanced glucose consumption, level of lipid and DNA synthesis, and lactate productions, indicating



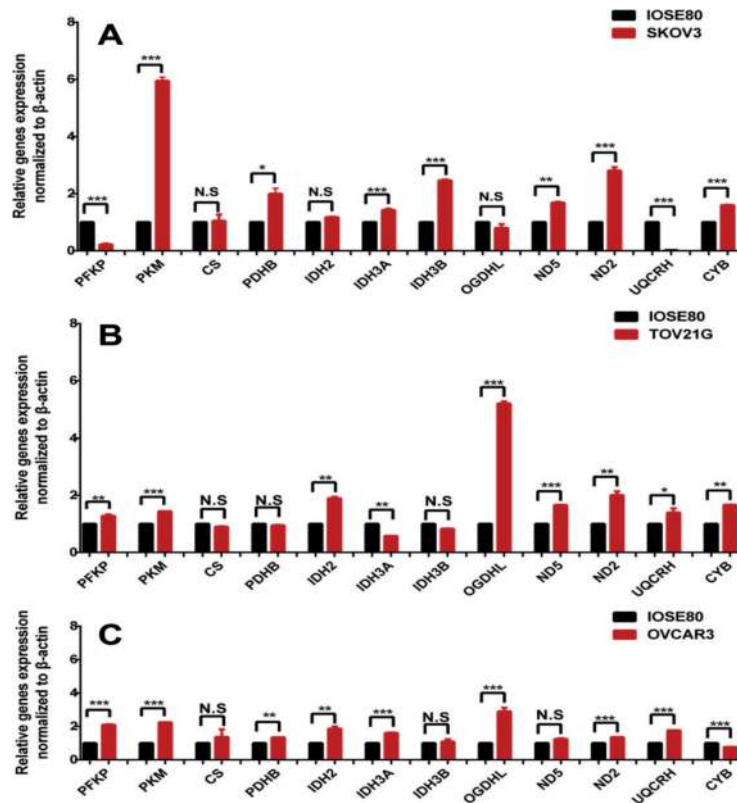
**Figure 2.**

The changes of key proteins in oxidative phosphorylation, Krebs cycle, and glycolysis pathways. A. The network derived from identified genes and pathways. The square box was pathways. The circular box was DEPs. The edge was the gene-gene and gene-pathway interactions. The node color from green to red was the gene expression level from low to high. The edge color was the correlation level from low to high (gray = 0.70–0.90, black = 0.90–1.00). B. Oxidative phosphorylation, Krebs cycle, and glycolysis pathways were altered in ovarian cancers. Reproduced from Li et al. [29], with permission from Elsevier, copyright 2018.

that PKM2 transformation promotes the Warburg effect [32]. Then, a novel series of inhibitors were developed to anti-Warburg-effect drugs for cancer treatment. For example, erastin-like anti-Warburg agents prevent mitochondrial depolarization induced by free tubulin and decrease lactate formation in cancer cells [33]. However, Warburg effect also has some limitations, because it completely ignored these facts that cancer cells had a great interaction with tumor microenvironment.

In the 2009, a new model for understanding the Warburg effect was proposed in tumor energy metabolism. The hypothesis is that cancer cells induce the aerobic glycolysis in neighboring stromal fibroblasts. These cancer-associated fibroblasts (CAFs) secrete energy-rich substances, including lactate and pyruvate, to tumor microenvironment. These energy-rich metabolites were eaten up by adjacent cancer cell and used by mitochondrial TCA cycle, resulting in a higher energy producing capacity. It termed this new idea as the “reverse Warburg effect” [10]. Taken all together, the reverse Warburg effect is a new energy metabolic pattern identified between cancer cells and CAFs, but this novel pattern does not deny Warburg effect status and still cannot replace it. Actually, the reverse Warburg effect extends energy metabolism content, which explained the nature of the heterogeneity and plasticity of cancer metabolism [34]. Although it's validated that the ‘reverse Warburg effect’ can be initiated by oxidative stress in two compartment metabolic coupling and change of cellular electromagnetic field, detailed mechanisms remain still unclear.

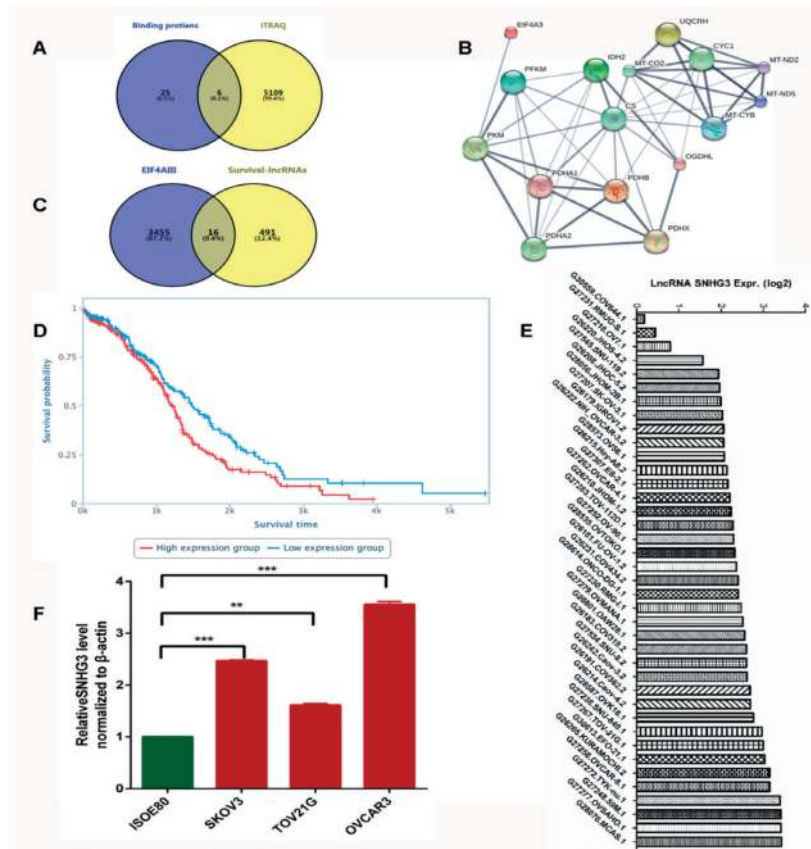
In order to verify the above of views, each EOC cell line (SK-OV3, TOV-21G, and OVCAR-3) showed high expression of energy metabolism-related genes relative to control cells IOSE80 by qRT-PCR, such as PKM, PDHB, IDH3A, IDH3B, ND5, ND2, and CYB in EOC cell lines relative to IOSE80 (**Figure 3**).



**Figure 3.** The gene expression changes of key proteins in glycolysis, Krebs cycle, and oxidative phosphorylation pathways confirmed by qRT-PCR analysis in ovarian cancer cells. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . N.S., non-significance. Reproduced from Li et al. [29], with permission from Elsevier, copyright 2018.

### 3.2 SNHG3 was significantly related to EOC survival through the key molecules in the energy metabolism pathways by their RNA-binding proteins or miRNA

Among identified 1198 mitochondrial DEPs and 205 tissue DEPs, PFKM, PKM, PDHB, CS, IDH2, IDH3A, IDH3B, OGDHL, ND2, ND5, CYB, and UQCRRH were enriched in glycolysis, Krebs cycle, and oxidative phosphorylation pathways. Six RNA-binding proteins (EIF4AIII, IGF2BP2, C22ORF28, UPF1, SFRS1, and EWSR1) were the iTRAQ-identified proteins in ovarian cancers (**Figure 4A**) based on Starbase v2.0 database. However, only EIF4AIII was associated with energy metabolic pathway, when did protein-protein network by STRING 10.0 software (**Figure 4B**). Furthermore, overlapping analysis between survival-related lncRNAs of EOC and lncRNAs binding with EIF4AIII obtained 16 lncRNAs (LINC00517, SNHG3, LBX2-AS1, ZNRF3-AS1, LINC00565, AL109767.1, WWTR1-AS1, HCG15, LEMD1-AS1, PDCD4-AS1, KIF9-AS1, SOS1-IT1, STARD13-IT1, PLCH1-AS1, ZNF674-AS1, and HOXC-AS3) existed those two groups. Among those 16 overlapped lncRNAs, only lncRNA SNHG3 was associated with energy metabolic pathways by GSEA analysis (**Figure 4C** and **D** and **Figure 4A** and **B**). The expression levels of lncRNA SNHG3 in different ovarian cancer cell lines indicated that poorly differentiated cell lines existed high SNHG3 expression, such as TYK-nu ovarian cancer cell line (**Figure 4E**). Additionally, q-PCR data demonstrated that SNHG3 was upregulated in SKOV3, TOV21G, and OVCAR3 relative to control cell line (IOSE80) (**Figure 4F**).



**Figure 4.** SNHG3 was significantly related to ovarian cancer survival through the key molecules in the energy metabolism pathways by their RNA-binding proteins or miRNA. **A.** Overlapping analysis of identified proteins and RNA binding proteins. **B.** Target DEPs-based protein-protein interaction network (STRING 10.0). **C.** Overlapping analysis of EIF4AIII-binding lncRNAs and lncRNAs involved in ovarian cancer survival. **D.** Kaplan-Meier survival analysis based on ovarian cancer SNHG3. **E** and **F.** SNHG3 expressions in ovarian cell lines. \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . Reproduced from Li et al. [29], with permission from Elsevier, copyright 2018.



One should also notice that gene sets were also significantly enriched in pathway of tamoxifen response (**Figure 5**), which indicated SNHG3 was associated with drug sensitivity or multidrug resistance. The comprehensive evaluation on SNHG3 may lead to ways to improve drug sensitivity of tamoxifen in EOCs.

Gene sets enrichment analysis showed that mRNA metabolism and 3'UTR-mediated translational regulation (**Figure 6**). Overlap analysis of RNAs-RNAs interaction networks showed that SNHG3 may regulated PDHB through binding hsa-miR-186-5p or hsa-miR-590-3p (**Figure 7A**), especially, hsa-miR-186-5p obtained high stringency to target PDHB with Starbase 2.0 analysis. Meanwhile, two binding sites were predicted between putative hsa-miR-186-5p and PDHB 3'UTR with RNAhybrid database (**Figure 7B and C**). Here, it can be forecasted boldly that SNHG3 might regulate the EOC energy metabolism by binding EIF4AIII and hsa-miR-186-5p, functioned as efficient sponges to regulate energy metabolism pathways though mitochondrial key molecules (**Figures 7D and 8A and B**).

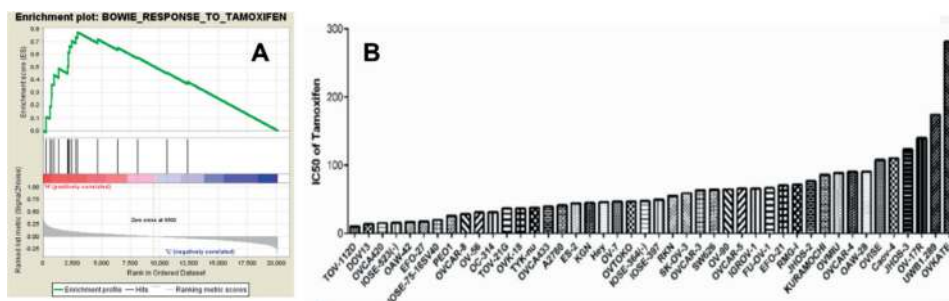
To further verify that SNHG3 can lead to the carcinogenesis in vivo, SKOV3 cells were transfected with either si-SNHG3 or a si-RNA negative control. Target genes, including PFKM, PKM, PDHB, CS, IDH2, IDH3A, IDH3B, OGDHL, ND5, ND2, CYB and UQCRH turned significant decrease expression (**Figure 9**). The results were further validated to a reasonable degree by Western blot (**Figure 10**).

Non coding RNAs, as one of epigenetic regulation form, play an important role in activation and suppression in a tumor by altering cell energy metabolism or biological behaviors [35]. However, lncRNAs have been identified and reported to be related to many kinds of carcinomas, little is known about lncRNAs whole molecular mechanisms in tumor energy metabolism. Recently, discovery of novel biomarkers focuses on ncRNAs, such as miR-125a, MALAT1, let-7a, miR-196a, HOXA11-AS, and lncRNA FAL1 [36]. Some biomarkers have been verified consistency in both tissues and serum, which improved clinical application value to use in early diagnosis or monitoring patient prognosis [37]. A number of studies have shown that lncRNAs can play an important role in tumorigenesis and progression through a variety of mechanisms, such as binding transcription factor, acting as miRNA sponge, ceRNA (competing endogenous RNAs) [38].

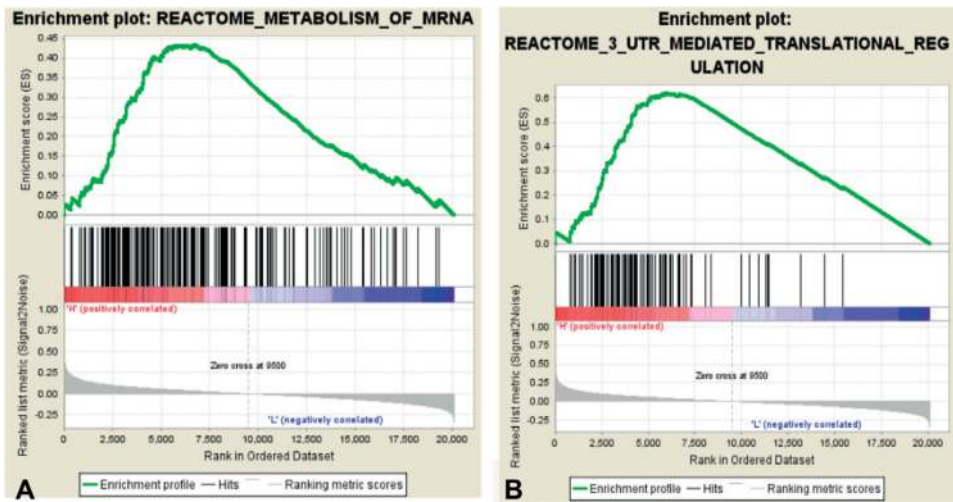
Therefore, lncRNA as an effective screening and their potential mechanisms in tumor energy metabolism would be rather influential in EOCs.

### 3.3 Potential therapeutic targets in metabolic symbiosis

Tumor tissues were made up by parenchymal cells and stromal elements. Parenchymal cells probably showed metabolism heterogeneity. So some cancer cells were high glycolytic cancer cells consisting with “Warburg effect”, while

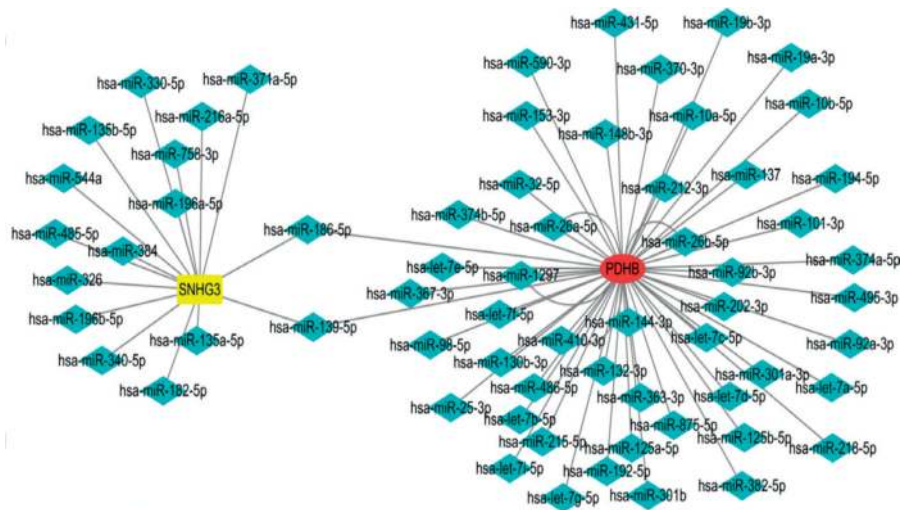


**Figure 5.** The results of SNHG3 by GSEA analysis revealed tamoxifen pathway. A. Genes were enriched in response to tamoxifen. B. IC<sub>50</sub> of tamoxifen in EOC cell lines. Reproduced from Li et al. [29], with permission from Elsevier, copyright 2018.



**Figure 6.**

The results of SNHG3 by GSEA analysis revealed mRNA metabolism and reactome 3'UTR-mediated translational regulation. A. Genes were enriched in metabolism of mRNAs. B. Genes were mainly enriched in reactome 3'UTR-mediated translational regulation. Reproduced from Li et al. [29], with permission from Elsevier, copyright 2018.

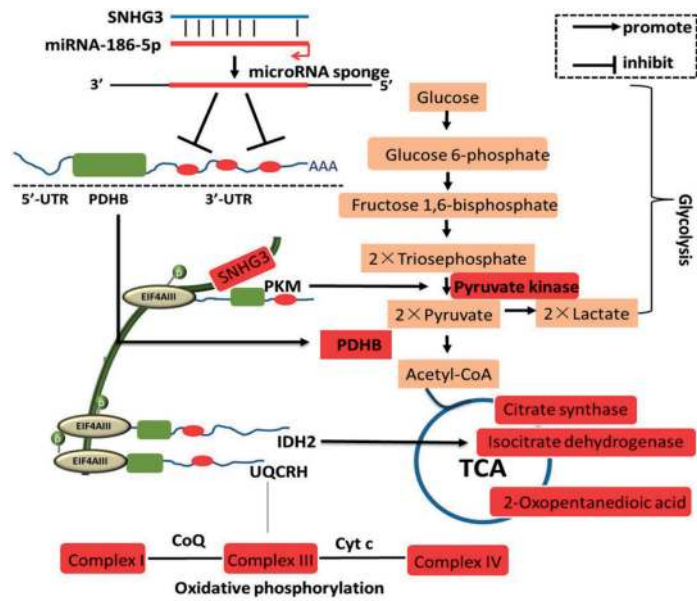


**Figure 7.**

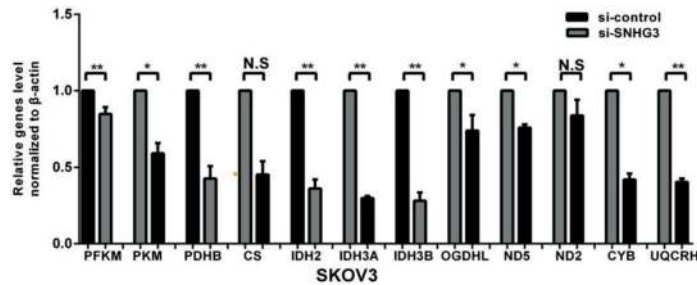
Overlapping analysis of the microRNA/mRNA and microRNA/lncRNAs interactive network. Reproduced from Li et al. [29], with permission from Elsevier, copyright 2018.

other cancer cells were oxidative cancer cells consisting with “reverse Warburg effect”. Cancer cells and stroma cells (especially CAFs) have metabolic symbiosis, so cancer cells induce oxidative stress of CAFs by secreting ROS to enhance aerobic glycolysis of CAFs. In turn, CAFs produced lots of nourishment to be ‘eaten’ up by cancer cells for producing ATP through Krebs cycle and oxidative phosphorylation [13].

MCT-1 and MCT-4 were overexpressed in EOC cells by qRT-PCR experiments, including SKOV3, TOV21G and OVCAR3 (**Figure 11**). Even though tumors were characterized by metabolic heterogeneity, MCT-1 and MCT-4 were just like lactate shuttle between cancer cells and stroma cells. The nanomaterial-siRNAs of SNHG3 might be promising for EOC patients to block the abnormal energy metabolism (**Figure 12**).



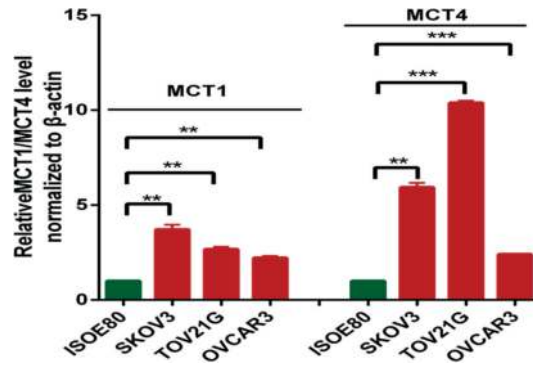
**Figure 8.** Schematic model of the potential signaling mechanisms between SNHG3 and energy metabolism in the EOC regulation. Reproduced from Li et al. [29], with permission from Elsevier, copyright 2018.



**Figure 9.** The mRNA expression levels of target genes of SNHG3 in EOC cells were determined by qRT-PCR. \* $p < 0.05$ , \*\* $p < 0.01$ . N.S., non-significance. Reproduced from Li et al. [29], with permission from Elsevier, copyright 2018.

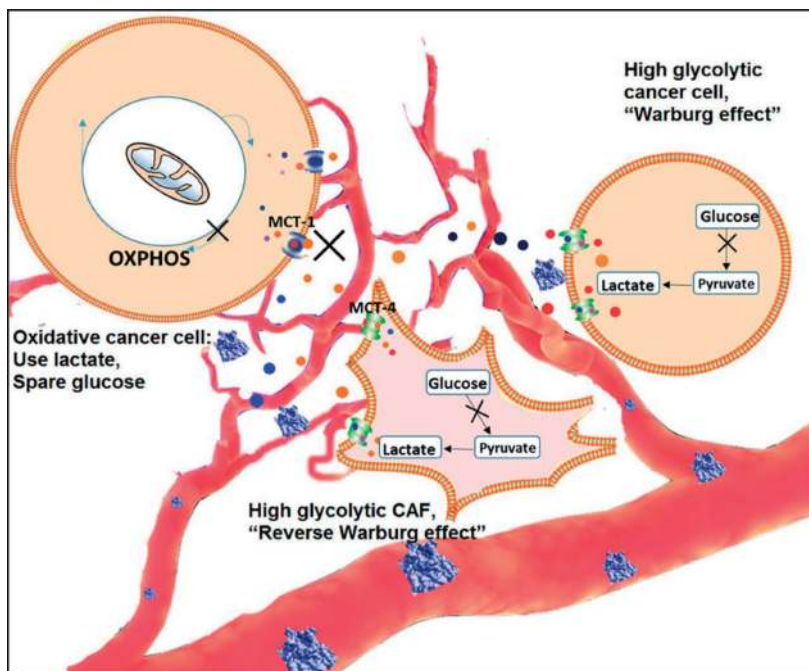
	Control	si-SNHG3	WB	P-value
	409074 ± 3047	421394 ± 3893	Ratio(si/nc)	
Glycolysis	PFKM	64049 ± 13945	1.01	0.816
	PKM	83997 ± 14188	0.86	0.003
Krebs cycle	PDHB	55873 ± 10965	0.53	0.005
	CS	77095 ± 9387	0.94	0.900
	IDH2	109793 ± 9559	0.68	0.006
	OGDHL	82176 ± 5430	—	—
ETC	UQCRH	42011 ± 3428	0.72	0.032
	β-actin	27297 ± 3708	—	—

**Figure 10.** The protein expression levels of target genes of SNHG3 in EOC cells were determined by Western blot. Reproduced from Li et al. [29], with permission from Elsevier, copyright 2018.



**Figure 11.**

The expression levels of MCT-1 and MCT-4 in EOC cells were determined by qRT-PCR. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . N.S., non-significance. Reproduced from Li et al. [29], with permission from Elsevier, copyright 2018.



**Figure 12.**

Energy metabolic heterogeneity-based potential therapeutic targets model. Parenchymal cells demonstrated energy metabolic heterogeneity. Some cancer cells showed the “Warburg effect” with highly glycolytic functions, and other cancer cells showed the “reverse Warburg effect” with oxidative cancer cells. The metabolic symbiosis existed between tumor cells and CAFs through MCTs. The RNA interference sequence of SNHG3 might be effective. Modified from Li et al. [29], with permission from Elsevier, copyright 2018.

#### 4. Conclusions

The identified 1198 mitochondrial DEPs, 205 tissue DEPs, and TCGA data in ovarian cancers provide new insights into human ovarian cancers, particularly the energy metabolism heterogeneity that ‘Warburg effect’ and ‘reverse Warburg effect’ were coexisted in ovarian cancer tissues. It emphasizes the important scientific merit in identity of new useful biomarkers within EOC energy metabolism heterogeneity system for the diagnosis and prognosis of ovarian cancer, and discovery of some potential therapeutic targets in energy metabolic interactions. Moreover, SNHG3 was related to energy metabolism through regulating hsa-miR-186-5p or RNA binding protein EIF4AIII, and those two molecules had target

sites with key proteins in TCA cycle and oxidative phosphorylation pathways (PDHB, IDH2, and UQCRH). Therefore, energy metabolism-based target treatments might be very promising for ovarian cancer patients to block both ‘Warburg effect’ and ‘reverse Warburg effect’.

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## **Conflict of interest**

We declare that we have no financial and personal relationships with other people or organizations.

## **Author’s contributions**

N.L. prepared figures, and wrote manuscript draft of the book chapter. X.H.Z. participated in writing and revising manuscript. X.Z. conceived the concept, designed the book chapter, prepared figures, wrote and critically revised the book chapter, coordinated and was responsible for the correspondence work and financial support.

## **Acronyms and abbreviations**

CAFs	cancer-associated fibroblasts
DEPs	differentially expressed proteins
ETC	electron transfer chain
iTRAQ	isobaric target for relative and absolute quantification
LC	liquid chromatography
lncRNAs	long non-coding RNAs
MCTs	mono-carboxylate transporters
mRNAs	messenger RNAs
MS/MS	tandem mass spectrometry
mtDEPs	mitochondrial differentially expressed proteins
ncRNAs	non-coding RNAs
NOS	nitric oxide synthase
OXPPOS	oxidative phosphorylation
SCX	strong cation exchange
TME	tumor-microenvironment

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