Open Access Contents
Int. J. Drug Dev. & Res.
| January - March 2015 | Vol. 7 | Issue 1 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

In Silico Comparative analysis of Cancer and Stem Cell Microarray data to demonstrate Molecular Transitions and the relative involvement of Glycolysis and Oxidative Phosphorylation cost-effective correlation of Bioenergetics and differentiation Processes -Applications In Drug Development

Ayyappa Kumar S. K

Suresh P. K.*

Bio Molecules & Genetics Division, School of Bio Sciences and Technology, VIT University Vellore, Vellore Dt. PIN:632014 (Tamil Nadu, India).

Corresponding Authors:
email: p.k.suresh@vit.ac.in; indian.ethos@gmail.com
Mobile: 9444047684

Abstract:
In the present study, we have validated the molecular switching between Glycolysis and Oxidative phosphorylation (OXPHOS) in certain cell types using open access *in silico* tools. Most of the cancers cells predominantly show higher rate of glycolysis (pyruvate formation) followed by lactic acid fermentation, in contrast with the predominant role of mitochondria in non- cancerous cells. This type of molecular switching is also noticed during the early stages of embryonic development, wherein the metabolic shift from OXPHOS to glycolysis occurs. Hence, it is necessary to study the switching between these pathways to most accurately replicate physiological conditions *ex vivo*/*in vitro* in differentiation/targeted differentiation-based studies. Further, an approach of this nature can help in further documenting the role of aberrations in cellular bioenergetics, contributing to neoplasia. A set of microarray datasets were selected from the NCBI GEO database, modelling three physiological conditions i.e., early development (stem cell), cancer, and Induced pluripotent cells (iPSCs). Data was analyzed using the 'R' software with packages imported from Bioconductor. Our results confirm the aforesaid switches/metabolic transitions in the selected data sets representing key cell types at various stages in the differentiation pathway. To the best of our knowledge, this is the first study of its kind wherein an *in silico* approach has been adopted to document/demonstrate the cellular, molecular transitions in two major metabolic pathways with ramifications in basic biology and drug development.

Keywords: *In silico*; microarray; glycolysis; oxidative phosphorylation; Gene Expression Omnibus database (GEO), Oxidative Phosphorylation (OXPHOS), Gene Ontology (GO), Gene Set Enrichment Analysis (GSEA).

Introduction

There are primarily two sources for ATP production– critical energy that is utilized for cell proliferation and survival (Glycolysis and Oxidative phosphorylation). Glycolysis (series of 10 enzyme-catalyzed reactions), involves the conversion of glucose to pyruvate to produce 2 ATPs per molecule of glucose. Tri-carboxylic acid (TCA) or Krebs cycle uses the resultant glycolysis-derived pyruvate, in a sequential set of electron-donating reactions. These electrons are channelled to the respiratory chain in mitochondria, using NADH and $FADH_2$ as the electron carriers. The end result is the ATP-synthase-mediated production of 36 ATPs per glucose molecule, produced via proton flow (down the electrochemical gradient) favouring the energetically unfavourable reaction between ADP and Pi. Apart from ATP production, oxygen, the final electron acceptor is reduced to water. Under relatively hypoxic conditions, as in the case of the muscle tissue of an individual after prolonged exercise, anaerobic glycolysis may be the preferred route with the metabolism of pyruvate to lactic acid by lactate dehydrogenase [1][2]. Similarly, hypoxic conditions in the early embryonic stages cause a metabolic shift from oxidative phosphorylation to glycolysis. However, oxidative phosphorylation is fully reinstituted upon preimplantation [3]. Such a relatively higher glycolysis-dependency, has also been observed in the case of the Inner Cell Mass (ICM), correlatable

with spherical, depolarized, immature peri-nuclearly localized mitochondria with low oxygen consumption, in contrast with those seen in the case of the Trophoectoderm [4][5][6]. Human embryonic pluripotent stem cells differentiation is accompanied by the acquisition of a more mature mitochondrial phenotype and a metabolic transition from glycolysis to OXPHOS [6][7][8][9]. This finding, corroborates the majority view of several authors, in terms of hypoxia being beneficial for the maintenance of hESCs in a pluripotent state [10,11] with reprogramming efficiency being favoured, under relatively high hypoxic conditions (low O_2 tensions), in both mouse and human somatic cells [12].

It is known that glycolysis and OXPHOS usually work in a coordinated manner, and the latter pathway produces around 15 times more ATPs per molecule of oxidized glucose than the former [13]. The conversion of glucose to glucose-6-phosphate by hexokinases is the one of the major rate-limiting steps in glycolysis, apart from membrane transport. Among other factors, Increased hexokinase activity, HK compartmentalization and amount of inhibitors are important regulators [14]. Further, the end result of the aforesaid 10 enzyme-catalysed reactions culminates in the production of two molecules each of pyruvate, NADH and ATP. Subsequently, pyruvate enters the TCA and is converted to acetyl coenzyme-A (source of reducing agents for the ETC-mediated ATP synthesis). This Krebs cycle intermediate links glycolysis with the OXPHOS pathway [15]. On the other hand, a relatively hypoxic micro-environment (wherein low O_2 tensions prevail, and/or the presence of dysfunctional mitochondria) will promote lactate production from pyruvate [16]. One of the important mitochondrial matrix enzyme complexes is the pyruvate dehydrogenase (PDH) complex, and this enzyme provides an important link between glycolysis and the TCA cycle. The enzyme has 3 subunits (E1, E2 and E3) and several copies of this enzyme are involved in the irreversible decarboxylation of pyruvate to acetyl-coenzyme A and NADH. Phosphorylation of the E1-α subunit, by one of the four pyruvate dehydrogenase kinase isoforms (PDHK1-4), leads to the complex-being inactivated. Conversely, removal of one phosphate group by one of the two pyruvate dehydrogenase phosphatases (PDP1 and PDP2) activates the complex [17]

Earlier studies have confirmed that glycolysis uptake increases during the morula stage. It was studied in morula, that glucose is required to express the glucose transporter GLUT3 which is essential for blastocyst formation and is also responsible for the expression of other monocarboxylate transporters [18,19]. In pluripotent stem cells, the hexokinases and lactate dehydrogenases are highly expressed and their activity is the highly rate-limiting reactions in glycolysis. These genes are further controlled by the pro-survival mTOR and PI3K pathways [20]. In cancer cells, the high consumption of ATP, pyruvate conversion to acetyl CoA by PDH and entry in to mitochondria, is blocked in hypoxic cancer cells by hypoxia inducible factor 1A (HIF1A) [21]. The metabolic shift between glycolysis and oxidative phosphorylation in stem cells, cancer cells and Induced pluripotent stem cells [22] microarray data were studied to verify the experimentally-determined differentially expressed gene [23]. Our *in silico* analysis has demonstrated that glucose transporters, hexokinase, pyruvate dehydrogenese as well as the pyruvate kinases are expressed highly in stem

cells, cancer cells and pluripotent stem cells. Based on the oxygen consumption rate, the expression of the oxidative phosphorylation genes are differentially expressed in stem cells, iPSCs as well as in the early embryonic stages. Due to the underdevelopment of mitochondria before the pre-implantation stage, the oxidative phosphorylation genes are less expressed. Our study, for the first time, provides an *in silico* strategy for better understanding molecular transitions in glycolysis and OXPHOS in certain cell types with ramifications in drug/small molecule development.

Materials and Methods

A set of microarray data sets were selected from the NCBI GEO repository http://www.ncbi.nlm.nih.gov/geo/. The accession IDs of these datasets are GSE18290 [24], GSE35373 [25], GSE13834 [26], GSE15744 [27] and GSE26910 [28]. These data sets represent gene expression patterns in three physiological conditions at the cellular level (early embryonic stem cells, Cancer cells and iPSCs).

Statistical Analysis:

Microarray data analysis was performed using the R statistical software. The microarray analysis packages were imported from the Bioconductor website. The affymetrix datasets were analyzed with the packages like 'affy'[29], 'genefilter' [30] 'simpleaffy'[31]. The Affymetrix datasets were normalized using the RMA algorithm. This algorithm performs probe summarization, background correction and normalization (lowess normalisation). Later, the normalized data was subjected to quality control analysis by filtering the data (based on the expression values) on expression using the 'simpleaffy' and 'genefilter' packages. The experiment grouping information was done based on the respective experimental design. Based on the sample grouping, statistical analysis was performed using the 'limma' [32] package by fitting the models. The false positives and negatives were removed using the Benjamini Hochberg FDR multiple testing correction method ($p < 0.05$) to identify statistically significant data.

Biological Contextualization:

Biological relevance of the statistically significant data was evaluated by retrieving the annotations from the **D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery (DAVID) [33, 34] and R. Later, the data was scanned for the biological relevance using the Gene Ontology (GO) [35] and Gene Set Enrichment analysis (GSEA) [36]. Our study mainly focused on metabolic shifts between Glycolysis and Oxidative phosphorylation in early embryonic development, cancer progression and stem cells. The entities involved in these two pathways were taken from the Wiki pathways. The overlapping/distinct, significantly altered genes (involved in the above two pathways) in the different microarray expression datasets, were converted into a Venn diagram, using the Venny online software application. Gene Set Enrichment Analysis (GSEA) was performed on the statistically significant gene list on the c1, c2, c3, c4, c5, c6, c7gene sets using GSEA software, the following gene setsKEGG_Oxidative_Phosphorylation,KEGG_Citrate_Cycle_TCA_Cycle,BIOCARTA_Glycolysis_pathway, KEGG_Pyruvate_metabolism were obtained at a enrichment factor of 0.3 to 0.4 and at p-value of 0.05. The above results show that genes involved in glycolysis and oxidative phosphorylation are highly enriched at different stages of embryonic

development, cancer progression and also in induced pluripotent stem cells.

Results and Discussions

The bioenergetic studies can provide a better understanding of their regulatory role in stem cells. Hence, two major metabolic pathways were selected to better understand their relative importance in stem cells as well as possibly provide overlapping/distinct bioenergetic snapshots that can be correlated with their differentiation status. Hence, we have selected the microarray datasets belonging to three physiological states (early embryonic stem cells, cancer cells and iPSCs). The GSE 18290, GSE35373, GSE 13834 represents data from stem cells and iPSCs. Differentially expressed significant genes were taken from the above datasets. Statistical analysis was done using limma at a p-value of 0.05.

In GSE 18290 data, the samples were divided in to six conditions (1st, 2nd, 4th, 8th, 16th and 32nd cell stages) based on their source. The expression pattern among the progressing cell stages were examined by performing fold change analysis on the statistically significant data, using the widely accepted 2-fold cut off. Analysis of microarray data showed high expression of glycolysis-related genes (8-cell, 16-cell and 32-cell stages), subsequent to their down-regulation at the earlier stages (2 and 4 cell stages). Aldolase A, a glycolysis enzyme, is upregulated in the 16 and 32 cell stages. All the three isoforms of Enolases, involved in glycolysis, at different of development are upregulated. The observed upregulation of Lactate Dehydrogenase (LDHA and LDHB) and the downregulation of testis-specific LDHC Genes again underscores their importance in anerobic glycolysis. Despite the up-regulation of malate dehydrogenase, involved in the of malate oxaloacetate shuttle, the down-regulation of the upstream enzyme (Glutamic-oxaloacetic transaminase 2) indirectly provides evidence of the glycolysis predominating. The up-regulation of the enzymes that are part of the pyruvate dehydrogenase complex (PDHA1 & PDHB) as well as Dihydrolipoamide S-acetyltransferase (DLAT-E2 component of the complex) indicates the progression towards the citric acid cycle. Also, upregulation of PDHX also indicates a similar role for this enzyme, since mutations in this enzyme have been linked to lactic acid build up. The down-regulation of Mitochondrial pyruvate carrier 1 (MPC1) or (BRP44L) indicates that the transport of pyruvate to this organelle is blocked, thereby providing inferential evidence of shuttling of the pyruvate to lactate. Glucose transporters (SLC2A1, SLC2A3) as well as hexokinase (HK1 and HK2) were highly up regulated, showing its significance in the early events in glycolysis-mediated glucose metabolism. All these biochemical events points towards the increasing dependence on glycolysis and Krebs cycle, especially in the 16 and 32 cell stages (**Table 1A, Table2A; Figure 1A**). This *in silico* finding is consistent with the published report that 85% of glucose is utilized in the blastocyst stage by aerobic respiration, unlike the markedly lower level of this biomolecule being utilized in the early stages. However, this can possibly be explained by the reported 14-fold higher efficiency of aerobic respiration, compensating for the lower amounts of glucose being utilized. Earlier studies indicate that during the pre-blastocyst stage, metabolism is tightly controlled by rate limiting glycolysis enzymes hexokinase and phosphofructokinases1. Thus pyruvate and its analogs are the only carbon source for the

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., January-March 2015, 7 (1): 69-81

developing cells. Similarly, other cell types (primordial germ cells, oocytes and spermatocytes), representing early stages in the development process also use the aforesaid, same metabolic intermediates (secreted by Ovarian Follicle and Sertoli cells). Later, the pyruvate oxidation occurs in mitochondria through a series of biochemical reactions (Kreb's cycle and followed by Oxidative phosphorylation) [24]. The hypoxic cellular conditions in the pre-blastocyst stages as well as the relatively immature mitochondria favouring glycolysis/lactate production is substantiated/mirrored by our *in silico* data.

The majority of the genes **(Table 1B, Table 2B, Figure 1B)** involved in oxidative phosphorylation are also reported to be upregulated indicating some flexibility in energy metabolism. The upregulation of NDUFA5 & NDUFC1, at the 32 in comparison with the 16 cell stage, indicates the possible preferential transcript of these isoforms in the inner mitochondrial membrane. The NDUFB5 downnregulation possibly indicates redundancy in mitochondrial oxidative phosphorylation. Further, importance of this isoform can be gauged by the fact that obesity and high-fat diet (risk factors for diabetes) is associated with decreases, (among other enzymes that are part of complex 1) specifically in this isoform [37].

The upregulation of GOT2 (**Table 3A, Table 3B, Figure 2A, Figure 2B**) indicates that the mitochondrial upregulation of aspartate to oxaloacetate, with the possible activation of the oxaloacetate-malate shuttle. The increased expression of PGK1, DLAT (Conversion of pyruvate to acetyl co-A) as well as the increases in the conversion of phosphoenol pyruvate to pyruvate (PKM) and the pyruvate transport protein – BRP44L and BRP44) into the mitochondria, provides evidence for the predominant involvement of glycolysis and TCA cycle in the cardiomyocyte differentiated from human embryonic stem cells. The down-regulation of an important protein in Complex 1 (ND6) perhaps indicates that could be mutations in the nuclear background that can possibly compensate for deficiency of this protein [38]. Our analysis of microarray data of selected data sets has also reported the relative, up regulation of glucose transporters, hexokinases in the 16 and 32 early embryonic stem cell stages. These proteins/enzymes have been known to be required for fixing the glucose molecules and are also important in the rate-limiting initial steps of glycolysis. In order to maintain the pH, anaerobic respiration-derived lactic acid is expelled from the cell. The reconversion of lactate back to pyruvate can occur through lactate dehydrogenases and this can avoid the build-up of lactic acid. NADH dehydrogenase 6 is the gene which was common among all the physiological conditions which is the core enzyme of the Oxidative phosphorylation pathway. However, the preference for pyruvate, by mammalian oocytes and preimplantation embryos [39] [40] eliminates neither the need for aerobic or for anaerobic respiration. Both glycolysis and OXPHOS are active and is correlated anatomically with more compact mitochondrial cristae. Replication of mitochondrial DNA commences and an increased amount of glucose is consumed at this stage [41] [42]. These data suggest that there is flexibility in energy metabolism during both oocyte maturation and embryo preimplantation development, with a shift in the balance towards aerobic respiration during blastocyst development and implantation. More experimental data from mice show that mitochondrial respiration is dispensable for embryonic development and adequate

implantation, even though there may be defects subsequently [43] [44]. Removal of pyruvate was associated with a drastic reduction in the development of human embryos, to be contrasted with glucose and lactate elimination having little effect [45]. These data suggest that, anaerobic respiration is necessary and yet not sufficient, again suggesting that both pathways are required for optimal development [46] as well as in mammalian cells.

The differential upregulation of the nonneural ENO1 (**Table 3A; Table 3B, Figure 4A & 4B**) and the downregulation of the neuronal ENO2 is slightly at variance with the data presented in **Figure 1A**. However, more information is needed about the differentiation status (the equivalent stage of development) of the human embryonic cells (hESCs) to better explain this observed variance. The upregulation of PDK1 and PDHA1 (glycolytic and TCA-related enzymes) is consistent with glycolysis being important in hESCs as well as the most presumably dedifferentiated embryonal cancers. The redundancy of the enzymes can be inferred by the fact that the pathway is active despite the downregulation of PDHX. The downregulation of DLAT & PKM in hESCs, in comparison with the results obtained with hESCs, is ineresting and may be compensated for by the upregulation of LDHC (lactate as a source of energy) and hence needs further evaluation experimentally. The downregulation of GOT2 (a mitochondrial enzyme) and the relative upregulation of SLC2A1 provides evidence for glycolysis enzymes upregulated in hESCs. Other solute transporters like SLC2A3 could possibly be compensating for the downregulated SLC2A1 in embryonal cancers and CpS. The striking downregulation of most of the glycolysis enzymes, CpS is worthy of mention and is consistent with the reported upregulation of oxidative phosphorylation enzymes in differentiated cells. molecular switching between Oxidative phosphorylation to glycolysis is also seen during cellular conversion from differentiated cells to pluripotent stem cells. It is also noticed that promotion of glycolysis enhances iPSC reprogramming, where as pharmacological inhibition of glycolysis or stimulation of Oxidative phosphorylation impairs iPSC reprogramming – important statement [47][48][49]. Since Complex 1 contains 45 distinct, nuclear and mitochondrial subunits, the downregulation of some of the subunits (NDUAFB1) can be plausibly compensated for by the presence of other subunits with similar functions (NDUAFV1). Simlarly, in hESCs, NDUFB9 is compensated for by the presence of NDUFS1..

Conclusion:

According to our analysis, the cancerous cells show high expression of glycolysis genes showing a cellular transition from glycolysis to oxidative phosphorylation as illustrated by Warburg effect. Early embryonic stem cells (pre-blastocyst) has shown an initial decline in both glycolysis and oxidative phosphorylation during 2,4 and 8 cell stages and a progressive increase in expression of those genes from 16 and 32-cell stages. The early embryonic stem cell upon differentiation has shown a transition in their metabolism from oxidative phosphorylation to Glycolysis. In the dedifferentiated cells (IPSCs), the cells showed a high expression of genes involved in glycolysis followed by oxidative phosphorylation. However, in keeping with our *in silico* findings and the observed experimental data corroborating the flexibility/transitions in energy metabolism,

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., January-March 2015, 7 (1): 69-81

expression profiling and next generation sequencing data and metabolomics (for e.g., GC-MS, LC-MS, NMR) that mimic the *in vivo* situation would generate more molecular information that would help in further refining the predictability of the *in silico* bioenergetic signature with respect to glycolysis and oxidative phosphorylation. This approach can help in the identification/refinement of small molecules that can possibly replace all of the four retroviral-mediated transduction of genes (Yamanaka factors) important for the generation of patient-specific embryonic stem cell-like cells [50].

Table 1A: Important Glycolysis genes:

Gene Symbol	Description and Source
Aldolase A	Conversion of fructose-1,6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (Found in Developing Embryo)
Enolase 1	Converts 2-phosphoglycerate to phosphoenolpyruvate (Tumor Suppressor)
Enolase 2	Converts 2-phosphoglycerate to phosphoenolpyruvate (Neural tissue development)
Enolase 3	Converts 2-phosphoglycerate to phosphoenolpyruvate (muscle development, regeneration)
Lactate dehydrogenase B	Conversion of lactate and Pyruvate, NAD and NADH
Lactate dehydrogenase C	Conversion of lactate and Pyruvate, NAD and NADH (Testis Specific)
Phosphoglycerate kinase 1	Conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate (Tumor cells)
Malate dehydrogenase	Reversible oxidation of malate to oxaloacetate (Mitochondria) and the conversion of oxaloacetate to malate (malata oxaloacetate shuttle)
Pyruvate dehydrogenase 1 (PDHB, PDHX)	Conversion of pyruvate to acetyl-CoA and CO_2, and provides the primary link between glycolysis and the tricarboxylic acid (TCA) cycle
Solute carrier Family 3	Facilitated glucose transporter
Dihydrolipoamide S-acetyltransferase	Conversion of pyruvate to acetyl coenzyme A
Glutamic-oxaloacetic transaminase 2	Role in amino acid metabolism and the urea and tricarboxylic acid cycles
Hexokinase 1,2	Conversion of glucose to produce glucose-6-phosphate
Pyruvate kinase M	Transfer of a phosphoryl group from phosphoenolpyruvate to ADP
Mitochondrial pyruvate carrier 1 (BRP44L)	Transporting pyruvate into mitochondria

Table 1B: Important Oxidative Phosphorylation genes:

Gene Symbol (OXPHOS)	Description
ATP5B, ATP5F1, ATP5E, ATP5G1, ATP5G3,ATP5I, ATP5J, ATP5O, ATP5L, ATP5S	This genes encodes a subunit of mitochondrial ATP synthase. Mitochondrial ATP synthase catalyzes ATP synthesis, utilizing an electrochemical gradient of protons across the inner membrane during oxidative phosphorylation
ATP6AP1	This gene encodes a component of a multisubunit enzyme that mediates acidification of eukaryotic intracellular organelles. (Early Embryonic development)
ND6, NDUFA2, NDUFA5, NDUFA7, NDUFA9, NDUFA10, NDUFAB1, NDUFB2, NDUFB5, NDUFB6, NDUFB9, NDUFB10, NDUFC1, NDUFS1, NDUFS3, NDUFS6, NDUFV1, NDUFV3	This genes encodes protein is a subunit of the hydrophobic protein fraction of the NADH ubiquinone oxidoreductase (complex 1), the first enzyme complex in the electron transport chain located in the inner mitochondrial membrane

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., January-March 2015, 7 (1): 69-81

Table 2A: Regulation of Glycolysis in Early Embryonic Stem cells

Gene Symbols	[2] vs [1]	[4] vs [1]	[8] vs [1]	[16] vs [1]	[32] vs [1]
ALDOA	1.4	1.71	3.40	3.81	3.57
ENO1	1.14	1.60	4.08	8.87	9.18
ENO2	1.15	1.30	4.65	5.46	5.25
ENO3	-1.2	1.00	2.00	3.94	2.86
LDHB	-1.12	4.94	5.65	5.06	6.88
MDH2	-1.11	1.34	1.19	-1.88	4.67
PDHA1	-1.13	1.35	1.61	4.99	6.57
PDHB	-1.43	-1.04	2.06	2.99	4.41
PDHX	-1.70	-1.36	-3.13	-1.03	2.02
SLC2A3	1.05	1.21	5.51	5.77	6.85
DLAT	-1.10	-1.0	-1.71	2.46	4.39
GOT2	-1.04	-1.11	-2.62	-4.43	-1.17
HK2	1.06	1.39	1.57	3.91	2.80
LDHC	-1.41	-1.03	-2.21	-3.74	-4.57
SLC2A1	-1.03	1.07	1.85	2.17	5.29
BRP44L	-1.48	-1.13	-2.57	-3.99	-2.03
HK1	1.08	1.33	4.30	5.68	7.80
LDHA	1.17	1.51	4.69	5.47	6.76

Table 2B: Regulation of Oxidative Phosphorylation in Early Embryonic Stem cells

Gene Symbol	[2 day] vs [1 day]	[4 day] vs [1 day]	[8 day] vs [1 day]	[16 day] vs [1 day]	[32 day] vs [1 day]
ATP5B	1.05	1.16	1.27	1.19	3.72
ATP5E	-1.18	1.81	4.07	7.41	9.11
ATP5F1	-1.50	1.09	1.67	4.02	7.20
ATP5G1	1.13	1.24	2.35	2.29	6.12
ATP5G3	-1.24	-1.09	-1.05	1.18	2.15
ATP5I	1.11	1.50	2.45	3.70	4.59
ATP5J	-1.15	1.15	1.25	2.24	4.45
ATP5O	1.07	1.50	1.34	2.16	4.31
ATP6AP1	1.09	1.03	1.63	4.04	3.41
ND6	1.58	1.85	2.94	3.50	2.97
NDUFA2	-1.52	1.16	5.52	6.45	4.22
NDUFA5	-1.70	1.00	-1.71	-1.05	2.68
NDUFA7	1.03	1.15	1.50	1.94	2.38
NDUFA9	-1.04	1.23	1.90	5.44	7.28
NDUFAB1	-1.32	1.01	1.07	2.02	2.60
NDUFB10	1.19	1.64	4.63	2.70	5.45
NDUFB2	1.12	1.34	1.88	2.55	3.53
NDUFB5	-1.20	-1.12	-3.17	-3.55	-2.28
NDUFB6	-1.20	1.23	1.41	2.48	2.79
NDUFB9	-1.01	1.78	1.53	2.01	1.95
NDUFC1	-1.70	-1.08	-1.39	-1.05	2.21
NDUFS1	1.18	1.25	1.11	1.89	2.88
NDUFS3	-1.24	1.14	1.74	1.91	2.49
NDUFS6	-1.07	1.89	5.12	8.44	13.33
NDUFV1	1.14	1.33	1.95	3.15	3.71
NDUFV3	-1.00	1.01	1.41	2.22	4.17

Figure 1A

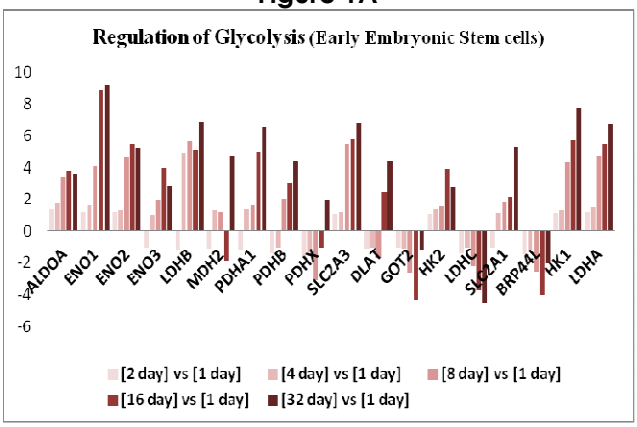


Figure 1B

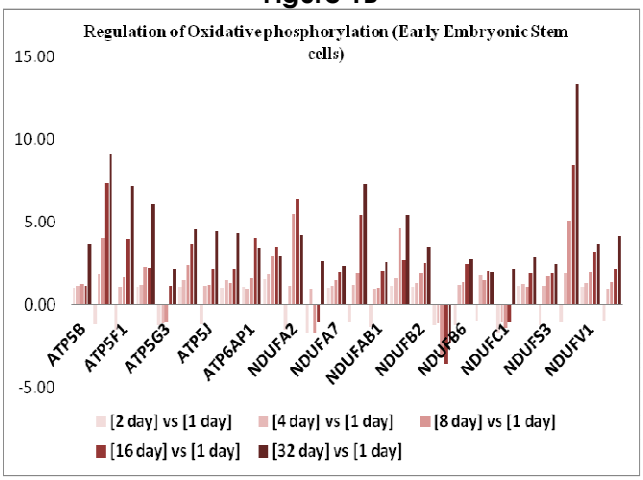


Figure 1A and Figure 1B: Regulation of Glycolysis and Oxidative phosphorylation genes (Early Embryonic Stem cells)**:** This graph represents the regulation (Up and down regulation) of glycolysis and oxidative phosphorylation genes differentially expressed during the early embryonic stages (Early ESC's), on y-axis the regulation of genes in the form of fold change was plotted and on x-axis the gene symbols were plotted. The microarray data was obtained from different cell stages of human early embryo (1, 2-cell, 4-cell, 8-cell, 16-cell and 32-cell). The gene regulations in different cell stages were compared against 1-cell stage, to study the gene expression patterns in developing cell stages.

Table 3A: Regulation of Glycolysis in (hESC's against hESC CM, beating EB, Fetal heart):

Gene Symbols	[Beating EB] vs [hESC]	[Fetal Heart] vs [hESC]	[hESC-CM] vs [hESC]
ENO2	1.81	2.36	1.34
PGK1	1.86	2.37	1.72
SLC2A3	2.65	3.45	2.55
DLAT	-2.19	-1.29	2.08
GOT2	2.94	1.34	2.02
HK2	-1.80	-1.90	-2.40
LDHC	1.53	-2.69	-1.48
SLC2A1	2.17	2.53	1.58
BRP44	2.64	2.75	3.58
BRP44L	2.53	3.89	4.28
LDHA	1.28	3.59	-1.04
PKM	1.11	2.44	2.64

Table 3B: Regulation of Oxidative phosphorylation in (hESC's against hESC CM, beating EB, Fetal heart):

Gene Symbol	[Beating EB] vs [hESC]	[Fetal Heart] vs [hESC]	[hESC-CM] vs [hESC]
ATP5E	2.37	5.37	2.06
ATP6AP1	1.41	2.72	2.43
ND6	-2.94	-2.13	-1.34
NDUFA10	2.01	3.50	1.00
NDUFA9	-1.14	2.34	1.19

Figure 2A-Regulation of Glycolysis genes (hESCs against hESC-CM, beating EB, Fetal Heart

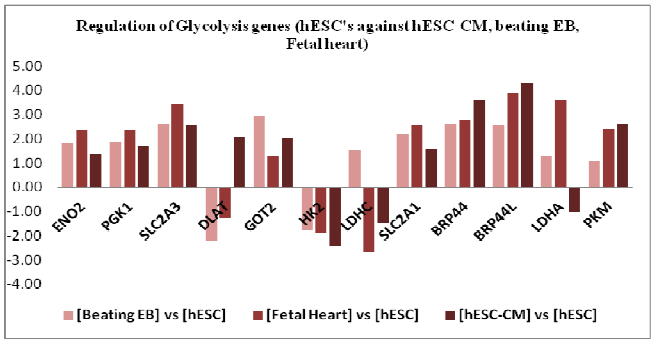


Figure 2B-Regulation of Oxidative Phosphorylation genes (hESCs against hESC-CM, beating EB, Fetal Heart

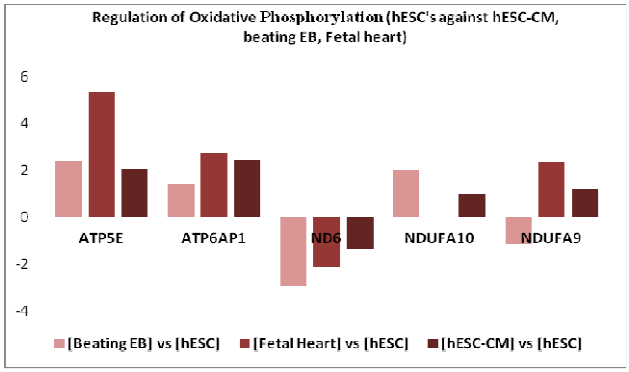


Figure 2A & Figure 2B: Regulation of Glycolysis and Oxidative phosphorylation genes (hESC's against hESC CM, beating EB, Fetal heart): This graph represents the regulation (Up and down regulation) of glycolysis and oxidative phosphorylation genes differentially expressed among three different physiological conditions they are: early embryonic stem cells, human embryonic stem cells-cardiomyocyte, fetal heart and beating heart. On y-axis the regulation of genes in the form of fold change was noted and on x-axis the gene symbols were plotted. The gene expression patterns of developing heart through hESC's to hESC's-CM, fetal heart and beating heart were studied.

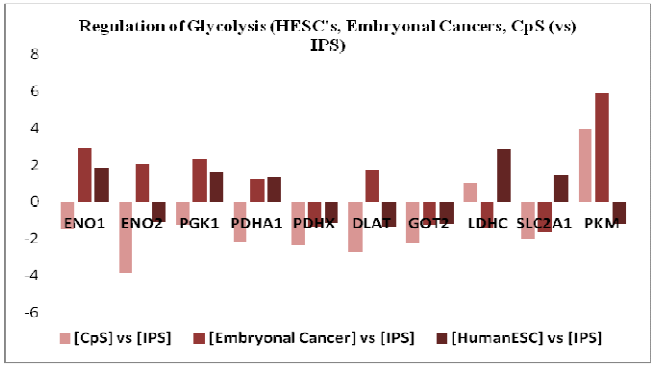
Table 4A: Regulation of Glycolysis in (hESC's, Embryonal Cancers, CpS (vs) IPS)

Gene Symbols	[CpS] vs [IPS]	[Embryonal Cancer] vs [IPS]	[HumanESC] vs [IPS]
ENO1	-1.46	2.90	1.80
ENO2	-3.85	2.02	-1.08
PGK1	-1.28	2.30	1.62
PDHA1	-2.14	1.25	1.39
PDHX	-2.35	-1.37	-1.13
DLAT	-2.71	1.76	-1.37
GOT2	-2.23	-1.21	-1.19
LDHC	1.05	-1.39	2.89
SLC2A1	-2.02	-1.63	1.48
PKM	3.98	5.88	-1.19

Table 4B: Regulation of Oxidative Phosphorylation in (hESC's, Embryonal Cancers, CpS (vs) IPS)

Gene Symbol	[CpS] vs [IPS]	[Embryonal Cancer] vs [IPS]	[Human ESC] vs [IPS]
ATP5G1	-2.47	1.31	1.85
ATP5S	-2.05	-1.45	1.63
ND6	-1.01	-2.78	2.63
NDUFAB1	-2.68	-1.00	-1.05
NDUFB9	-2.05	-1.16	1.03
NDUFS1	-2.01	1.53	-1.30
NDUFV1	1.08	3.49	1.28

Figure 3A: Regulation of Glycolysis genes (HESC's, Embryonal Cancers, CpS (vs) IPS)



Regulation 3B: Regulation of Oxidative Phosphorylation genes (hESC's, Embryonal Cancers, CpS (vs) IPSCs)

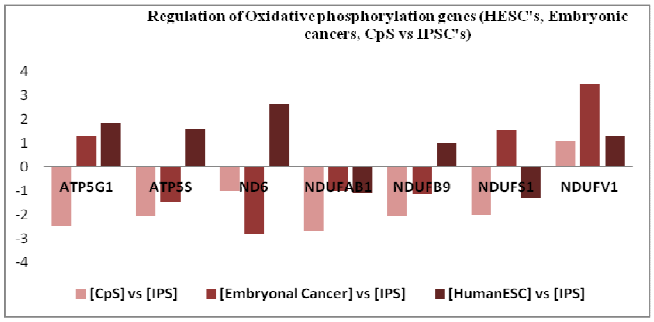


Figure 3A and Figure 3B: Regulation of Glycolysis and Oxidative phosphorylation genes (HESC's, Embryonal Cancers, CpS (vs) IPS): This graph represents the regulation (Up and down regulation) of glycolysis and oxidative phosphorylation genes differentially expressed among three different physiological conditions they are: early embryonic stem cells, embryonal cancers, and induced pluripotent stem cells. On y-axis the regulation of genes in the form of fold change was noted and on x-axis the gene symbols were plotted. The gene expression patterns of prostate cancer stromal cells, embryonal cancers and human embryonic stem cells were compared against gene expression patterns of Induced pluripotent stem cells.

Covered in Scopus & Embase, Elsevier
Int. J. Drug Dev. & Res., January-March 2015, 7 (1): 69-81

References:

1) Gatenby RA; Gillies RJ (2004). Why do cancers have high aerobic glycolysis Nature Reviews Cancer 4 (11).
2) Kim JW, Dang CV (2006). Cancers molecular sweet tooth and the Warburg effect. Cancer Res. 66 : 8927–8930.
3) Leese HJ, Barton AM (1984) Pyruvate and glucose uptake by mouse ova and preimplantation embryos. J Reprod Fertil 72: 9–13.
4) Ramalho-Santos J, Varum S, Amaral S, Mota PC, Sousa AP, Amaral A. (2009)Mitochondrial functionality in reproduction: from gonads and gametes toembryos and embryonic stem cells. Hum Reprod Update 15: 553–572.
5) Stern S, Biggers JD, Anderson E (1971) Mitochondria and early development of the mouse. J Exp Zool 176: 179–191.
6) Houghton FD (2006) Energy metabolism of the inner cell mass and trophectoderm of the mouse blastocyst. Differentiation 74: 11–18.
7) St John JC, Ramalho-Santos J, Gray HL, Petrosko P, Rawe VY, Navara CS, Simerly CR, Schatten GP (2005) The expression of mitochondrial DNA transcription factors during early cardiomyocyte in vitro differentiation from human embryonic stem cells.Cloning Stem Cells 7: 141–153.
8) Cho YM, Kwon S, Pak YK, Seol HW, Choi YM, Park do J, Park KS, Lee HK (2006) Dynamic changes in mitochondrial biogenesis and antioxidant enzymes during the spontaneous differentiation of human embryonic stem cells. Biochem Biophys Res Commun 348: 1472–1478.
9) Prigione A, Fauler B, Lurz R, Lehrach H, Adjaye J (2010) The senescence related mitochondrial - oxidative stress pathway is repressed in human induced pluripotent stem cells. Stem Cells 28: 721–733.
10) Ezashi T, Das P, Roberts RM (2005) Low O_2 tensions and the prevention of differentiation of hES cells. Proc Natl Acad Sci U S A 102: 4783–4788.
11) Forristal CE, Wright KL, Hanley NA, Oreffo RO, Houghton FD (2010) Hypoxia inducible factors regulate pluripotency and proliferation in human embryonic stem cells cultured at reduced oxygen tensions. Reproduction 139: 85–97.
12) Yoshida Y, Takahashi K, Okita K, Ichisaka T, Yamanaka S (2009) Hypoxia enhances the generation of induced pluripotent stem cells. Cell Stem Cell 5:237–241
13) Alberts B, Johnson A, Lewis J, Raff M, Roberts K, & Walter P (2002). Molecular Biology of the Cell. 4th edition. New York: Garland Science
14) Wasserman DH, Kang L, Ayala JE, Fueger PT, Lee-Young RS. (2011). The physiological regulation of glucose flux into muscle in vivo. J Exp Biol 214::254-62.
15) Butcher L, Coates A, Martin KL, Rutherford AJ & Leese HJ 1998 Metabolism of pyruvate in the early human embryo. Biology of Reproduction 58 1054–1056.
16) Adeva-Andany M, López-Ojén M, Funcasta-Calderón R, Ameneiros-Rodríguez E, Donapetry-García C, Vila-Altesor M, Rodríguez-Seijas J (2014). Comprehensive review on lactate metabolism in human health. Mitochondrion 17:76-100.
17) Holness MJ, Sugden MC (2003) Regulation of pyruvate dehydrogenase complex activity by reversible phosphorylation. Biochem Soc Trans 31: 1143–1151.
18) Chatot CL, Ziomek CA, Bavister BD, Lewis JL & Torres I An improved culture medium supports development of random-bred 1-cell mouse embryos in vitro(2003 Journal of Reproduction and Fertility 86:679–688.
19) Jansen S, Esmaeilpour T, Pantaleon M and Kaye PL (2006). Glucose affects monocarboxylate cotransporter (MCT) 1 expression during mouse pre-implantation development. Reproduction 131:469–479.

20) Ito K, Suda T (2014). Metabolic requirements for the maintenance of self-renewing stem cells. Nat Rev Mol Cell Biol 15:243-256.

21) Palsson-McDermott EM, O'Neill LA (2013). The Warburg effect then and now: from cancer to inflammatory diseases. Bioessays 35:965-73.

22) Marín-Hernández A, Gallardo-Pérez JC, Ralph SJ, Rodríguez-Enríquez S, Moreno-Sánchez R. (2009). HIF-1alpha modulates energy metabolism in cancer cells by inducing over-expression of specific glycolytic isoforms. Mini Rev Med Chem 9:1084-1101.

23) Folmes CD, Martinez-Fernandez A, Faustino RS, Yamada S, Perez-Terzic C, Nelson TJ, Terzic A (2013). Nuclear reprogramming with c-Myc potentiates glycolytic capacity of derived induced pluripotent stem cells. J Cardiovasc Transl Res 6:10-21.

24) Xie D, Chen CC, Ptaszek LM, Xiao S et al. (2010) Rewirable gene regulatory networks in the preimplantation embryonic development of three mammalian species. Genome Res 20:804-15.

25) Vêncio EF, Nelson AM, Cavanaugh C, Ware CB Milller DG, Garcia JC, Vêncio RZ, Loprieno MA, Liu AY.(2012). Reprogramming of prostate cancer-associated stromal cells to embryonic stem-like. Prostate 72:1453-1463.

26) Cao F, Wagner RA, Wilson KD, Xie X Fu JD, Drukker M, Lee A, Li RA, Gambhir SS, Weissman IL, Robbins RC, Wu JC (2008)Transcriptional and functional profiling of human embryonic stem cell-derived cardiomyocytes. PLoS One. 3:e3474.

27) Yi H, Xue L, Guo MX, Ma J, Zeng Y, Wang W, Cai JY, Hu HM, Shu HB, Shi YB, Li WX (2010).Gene expression atlas for human embryogenesis. FASEB J 24:3341-50.

28) Planche A, Bacac M, Provero P, Fusco C Delorenzi M, Stehle JC, Stamenkovic I (2011). Identification of prognostic molecular features in the reactive stroma of human breast and prostate cancer.PLoS One 6:e18640.

29) Gautier L, Cope L, Bolstad BM and Irizarry RA (2004). Affy-analysis of Affymetrix Gene Chip data at the probe level." Bioinformatics, 20:307–315. ISSN 1367-4803.

30) Gentleman R, Carey V, Huber W and Hahne F. genefilter: genefilter: methods for filtering genes from microarray experiments. R package version 1.46.1.

31) Miller CJ. simpleaffy: Very simple high level analysis of Affymetrix data. R package version 2.40.0,http://www.bioconductor.org,http://bioinformatics.picr.man.ac.uk/simpleaffy/.

32) Smyth GK (2005). "Limma: linear models for microarray data." In Gentleman R, Carey V, Dudoit S, Irizarry R and Huber W (eds.), Bioinformatics and Computational Biology Solutions Using R and Bioconductor, pp. 397–420. Springer, New York.

33) Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. Nature Protoc. 2009;4(1):44-57.

34) Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009;37(1):1-13.

35) The Gene Ontology Consortium. Gene ontology: tool for the unification of biology. Nat Genet. May 2000;25(1):25-9.

36) Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 102:15545-15550.

37) Sparks L.M., Xie H., Koza R.A., Mynatt R., Hulver M.W., Bray G.A., Smith S.R. (2005). A high-fat diet coordinately down-regulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. Diabetes. 54:1926-1933.

38) Deng J.H., Li., Park J.S., Wu J., Hu P., Lechleiter J., Bai Y. (2006). Nuclear suppression of

mitochondrial defects in cells without the ND6 subunit. Mol Cell Biol. 26:1077-1086.

39) Butcher L, Coates A, Martin KL, Rutherford AJ & Leese HJ (1998). Metabolism of pyruvate in the early human embryo. Biology of Reproduction 58 1054–1056

40) Bavister BD & Squirrell JM (2000). Mitochondrial distribution and function in oocytes and early embryos. Human Reproduction 15 (Supplement 2)189–198.

41) Houghton FD & Leese HJ (2004). Metabolism and developmental competence of the preimplantation embryo. European Journal of Obstetrics, Gynecology, and Reproductive Biology 115 S92–S96.

42) Aiken CE, Cindrova-Davies T & Johnson MH (2008). Variations in mouse mitochondrial DNA copy number from fertilization to birth are associated with oxidative stress. Reproductive Biomedicine Online 17 806–813.

43) Piko L & Chase DH (1973). Role of the mitochondrial genome during early development in mice. Effects of ethidium bromide and chloramphenicol. Journal of Cell Biology 58 357–378.

44) Larsson NG, Wang J, Wilhelmsson H, Oldfors A, Rustin P, Lewandoski M, Barsh GS & Clayton DA (1998). Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. Nature Genetics 18 231–236

45) Wilding M, Fiorentino A, De Simone ML, Infante V, De Matteo L, Marino M & Dale B (2002). Energy substrates, mitochondrial membrane potential and human preimplantation embryo division. Reproductive Biomedicine Online 5 39–42.

46) Wilding M, Di Matteo L & Dale B (2005). The maternal age effect: a hypothesis based on oxidative phosphorylation. Zygote 13 1–7.

47) Yoshida Y., Takahashi K., Okita K.,Ichisaka T., Ya manaka S. (2009). Hypoxia enhances the generation of induced pluripotent stem cells. Cell Stem Cell 5: 237-241.

48) Zhu S., Li W., Zhou H., Wei W., Ambasudhan R., Lin T., Kim J., Zhang K., Ding S (2010). Reprogramming of human primary somatic cells by OCT4 and chemical compounds. Cell Stem Cell 7, 651-655.

49) Folmes CDL, Nelson TJ, Martinez-Fernandez A, Arrell DK, Lindor JZ, Dzeja PP, Ikeda Y, Perez-Terzic C, Terzic A. (2011). Somatic oxidative bioenergetics transitions into pluripotency-dependent glycolysis to facilitate nuclear reprogramming. Cell Metab 14:264-271.

50) Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA (2008). Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol 26:795-97.

Article History: ----------

Date of Submission: 22-01-2015

Date of Acceptance: 09-02-2015

Conflict of Interest: NIL

Source of Support: NONE