Journal of Medical Genetics and Genomics Vol. 3(7), pp. 115 - 121, October 2011
Available online at http://www.academicjournals.org/JMGG
ISSN 2141-2278 ©2011 Academic Journals

Full Length Research Paper

Role of pyruvate dehydrogenase kinases (PDK's) and their respective microRNA's in human ovarian cancer

Sheema Sameen[1], Zoya Khalid[1] and Shaukat Iqbal Malik[2]*

[1]Department of Bioinformatics and Biotechnology, International Islamic University, Islamabad, Pakistan.
[2]Department of Bioinformatics, Muhammad Ali Jinnah University (MAJU) New Campus, Islamabad, Pakistan.

Accepted 13 October, 2011

Cancer is a metabolic disorder and in energy metabolism, pyruvate dehydrogenase kinases (PDKs) play a very vital role, which make them important candidate for involvement in cancer. The bioinformatics analysis performed on ovarian cancer data sets taken from gene expression Omnibus proved the up regulation of PDK2 and PDK4. The regulating microRNA's for these two genes were also predicted computationally, which were found down regulated in cancer and hence confirmed the over expression of PDK2 and PDK4. Further investigations on the behaviour of PDK's and their corresponding microRNA's will provide a major breakthrough in cancer research and investigation.

Key words: Pyruvate dehydrogenase kinase(PDK), metabolic disorder, ovarian cancer, microRNA.

INTRODUCTION

Cancer is a metabolic disorder (Seyfried and Shelton, 2007). In cellular biology, the mitochondria (known as the power house of the cell) is the organelle that is considered as a supreme commander for all of the metabolic processes going on in the human body (Stine, 1956). Otto Warburg was the first one to report the mitochondrial dysfunction in tumor cells (Warburg, 1956). His findings opened a new line of research and understanding for cancer. After Warburg, many work was carried out regarding it (Cavalli and Liang, 1998) and it was proved that tumor cell mitochondria has a disrupted energy metabolism rate as compared to the normal cell mitochondria (Mayevsky, 2009; Seyfried and Mukherjee, 2005; Chen et al., 2009; Ramanathan et al., 2005). So it is necessary to unravel the molecular mechanisms in glycolysis and citric acid cycle which is further involved in different cancers. In every cell, the link of glycolysis with citric acid cycle is maintained by an irreversible process of oxidative decarboxylation of pyruvate to form acetyl coA. This reaction is catalyzed by pyruvate dehydrogenase complex which plays a crucial role in this reaction. The glucose metabolism is regulated by pyruvate dehydrogenase kinase (PDK) which has the ability to switch off the mitochondrial pyruvate dehydrogenase complex. Four isoenzymes of pyruvate dehydrogenase kinase (PDK1, PDK2, PDK3 and PDK4) with tissue specific activities have been identified in mammals so far (Bowker-Kinley et al., 1998). The PDKs activity was mainly observed in case of fasting, starvation and in diabetes (Majer et al., 1998; Guerre-Millo et al., 2000). Increase in PDK4 activity in starvation and diabetes was shown in rat tissues by Wu et al. (1999). PDK2 is declared as a major isoenzyme responsible for regulation of pyruvate dehydrogenase complex (Gudi et al., 1995).

Although the major reported articles said that the contribution of these isoforms is mostly in muscles, heart and liver but due to the critical role of these isozymes in energy metabolism it is believed that any defect in their activity may become a cause for cancer. Tumor cells have increased energy demands and in order to fulfil it, they try to take up more oxygen and nutrients than normal cells, which create a hypoxic environment (Harris, 2002). Cancer cells undergo various adaptations in order to survive in unfriendly hypoxic environment. These adaptations are induced by Hypoxia-Inducible Factor (HIF) (Semenza, 2003; Gordan and Simon, 2007). HIF uses various different ways to regulate this mechanism and one of them is by switching off the conversion of pyruvate into acetyl coA by blocking the activity of PDH complex through directly stopping PDK1 activity (Kim et al., 2006). In the meanwhile, estrogen- related receptors (ERR) stimulate increase in PDK4 expression in order to prevent aerobic metabolism of glucose (Wende et al.,

*Corresponding author. E-mail: msiqbqlpd@yahoo.com.

2005; Araki and Motojima, 2006; Zhang et al., 2006). This mechanism allows the tumor cells to survive in hypoxic environment and fulfil the high energy demand of these cells (Ao et al., 2008). The crucial role of PDKs in survival of tumors is well understood by this mechanism. The increased expressions of PDK1 in breast cancer (Maurer et al., 2009; Lin et al., 2005) and decrease of PDK4 in cervical cancer (Carlson et al., 2007) also validates the presence of their pivotal role in cancers. The down regulation expression of PDK is just found in cervical cancer. It can be concluded that on the whole PDK4 expression is up regulating in a variety of cancers.

Ovarian cancer is the greatest gynaecological problem worldwide and it is believed to be a silent killer disease because of late stage diagnosis, which decreases the cure rate and increases the mortality. Discovery of microRNA's involvement in cancer pathogenecity has opened new insights in cancer research. The problem not just confines in the expression of those genes which regulates in case of cancer but there are some other microRNA genes which basically influence the expression of those regulated genes. In other words, the expression of those genes which are involved in cancer, basically depends upon some other genes known as microRNA genes. So, in order to fully understand the gene expression mechanism, it is necessary to study the targeting microRNA's expression along with the genes. The involvement of PDKs in human ovarian cancer has never been reported before. Here, in this article we ought to understand not only the involvement of PDKs in human ovarian cancer but also their targeting microRNAs to present the complete picture of the problem.

METHODS

Two ovarian cancer expression datasets were downloaded from Gene Expression Omnibus (GEO). These two datasets GSE4122 and GSE6008 contain expression amounts for both normal and malignant ovaries. GSE6008 contain 99 individual ovarian tumors and 4 individual normal ovary samples contributed by Hendrix ND and the second dataset GSE4122 is contributed by Tate and co workers with 32 cancerous tumors and 14 controls. These datasets were mined for finding novel gene markers in human ovarian cancer. At first, two statistical approaches were employed e.g. t-test and SAM (significance analysis of microarrays) in order to extract the most statistically significant genes from both datasets. T-test extracts genes on the basis of threshold criteria, with the p-value less then 0.05. The volcano plot displays the results of t-test. SAM digs out the genes on the basis of fold change. Fold change >2 was considered significant. SAM results were depicted in the form of SAM graph. This resulted in a shortlisted set of genes. We found two novel genes associated with ovarian cancer in our shortlisted data set, the pyruvate dehydrogenase kinase 2 (PDK2) and pyruvate dehydrogenase kinase 4 (PDK4). For further validation of results, in silico Serial analysis of gene expression (SAGE) (Boon et al., 2002) and northern blot analysis were performed, in which the expression of genes was calculated by counting the tags per million.

The results were also analyzed by GENT (Gene Expression across Normal and Tumor tissue) database (Shin et al., 2011). This is a very new database for microarray data analysis. It contains two data sets U133Plus2 and U133A. For prediction of targeting microRNA's of pdk2 and pdk4, we used four different bioinformatics target prediction tools namely TargetScan (Lewis et al., 2005; Grimson et al., 2007; Friedman et al., 2009), miRanda (Enright et al., 2003; John et al., 2005; Betel et al., 2010; Betel et al., 2008), Pictar (Grün et al., 2005; Krek et al., 2005; Lall et al., 2006; Chen and Rajewsky, 2006) and miRGen (Megraw et al., 2006). Then, the binding energies of predicted microRNA's were calculated. The microRNA binds with specific energy with their respective genes. This binding energy can be calculated by using RNAhybrid tool (Rehmsmeier et al., 2004; Krüger and Rehmsmeier, 2006) for finding the minimum free energy hybridization of mRNA of target gene and microRNA.

RESULTS

The statistical analysis of microarray datasets of ovarian cancer, exposed significant genes highlighted in the expression pattern. The t-test and SAM hauled the most reliable genes differentially expressed in the malignant and normal ovarian data sets. PDK2 and PDK4 were among those extracted genes. These two members of PDK gene family were never identified previously for their connection with human ovarian cancer. Up regulation of PDK2 and PDK4 in t-test was observed in both datasets, while in SAM of GSE6008 only PDK4 expression was seen as a significant gene while SAM declared pdk2 as a non significant gene because of its low fold change. The results of statistical tests are shown in the form of volcano plots (Figures 1 and 2) and SAM graphs (Figures 3 and 4). The significance of these genes was confirmed by their p-value and fold changes obtained from these tests (Table 1). Verification of PDK2 and PDK4 over expression in human ovarian cancer was done through SAGE, where both tissue and cell line libraries of normal and cancerous ovaries were scanned.

In cell lines, normally there is no expression of PDK2 and PDK4. PDK2 over expression verified in cancerous tissues and cell lines but PDK4 expression result was very different, as it is up regulated in case of cell lines but it shows no or little expression in tissues (Table 2). Similarly, when pdk4 was analyzed using GENT, it was noted that in U133Plus2 it was up regulated while in data set U133A its expression is down regulated. PDK2 over expression was also verified by both data sets of GENT. Targeting microRNA's was predicted by using various tools and common results from these softwares were taken only. The binding energies were also calculated and finally only those microRNA's were shortlisted which were common output of all prediction tools and whose binding energies were also minimum (Table 3).

DISCUSSION

The microarray analysis of ovarian cancer data sets resulted in a collection of genes that can be the potential targets for disease. The results revealed PDK2 and PDK4 as novel biological markers for ovarian cancer

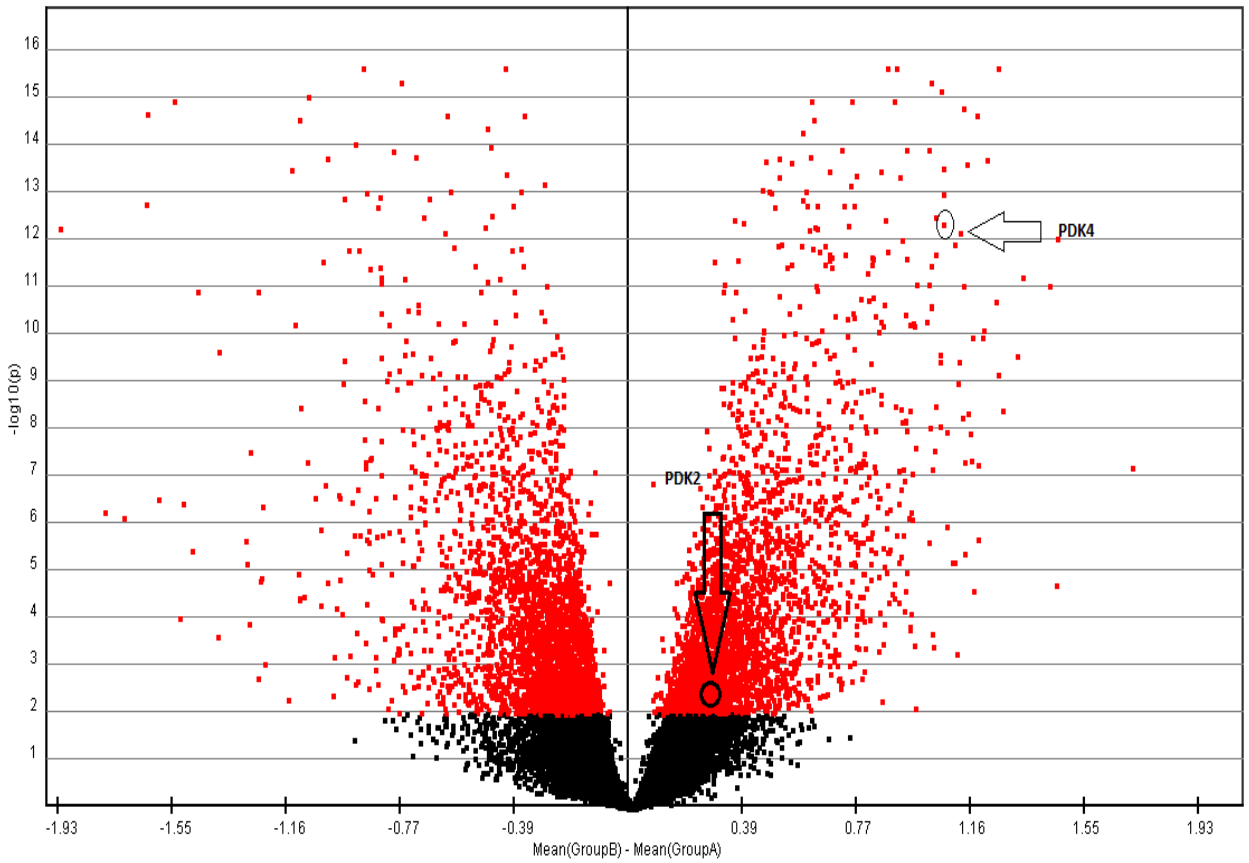

Figure 1. Volcano plot showing PDK2 and PDK4 differential expression in GSE6008.

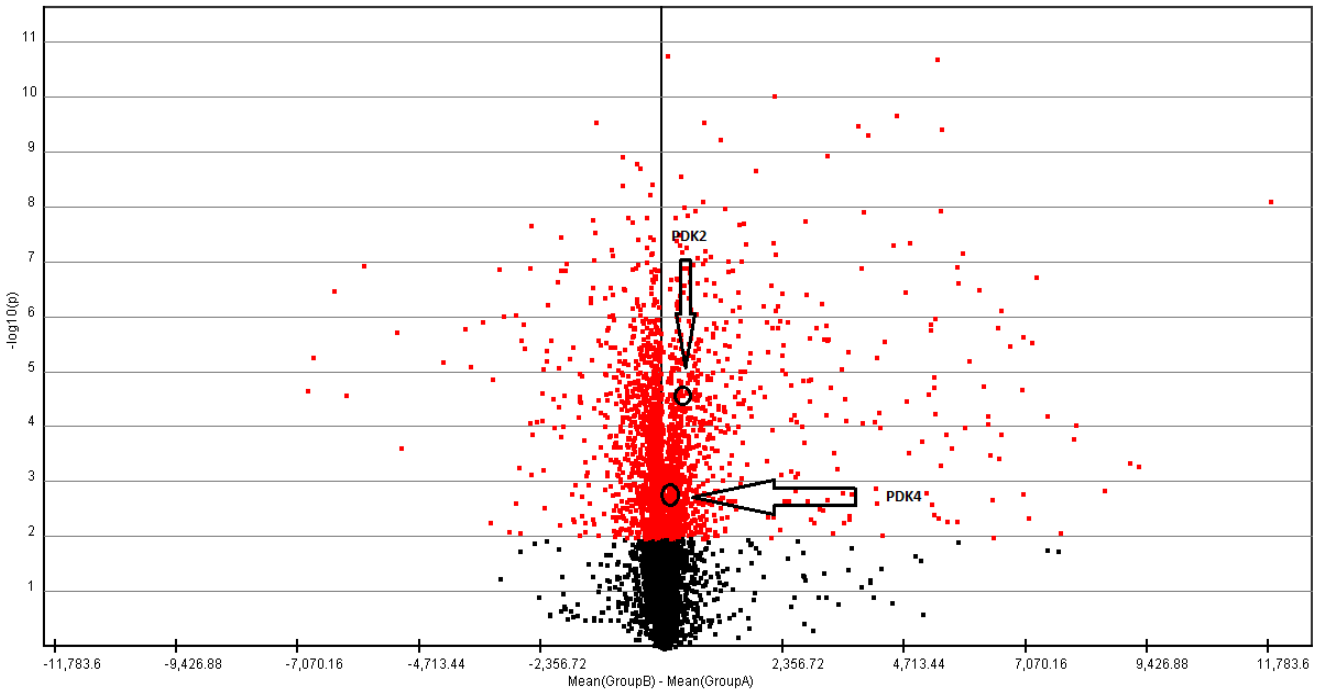

Figure 2. Volcano plot showing differential expression of PDK4 and PDK2 GSE4122.

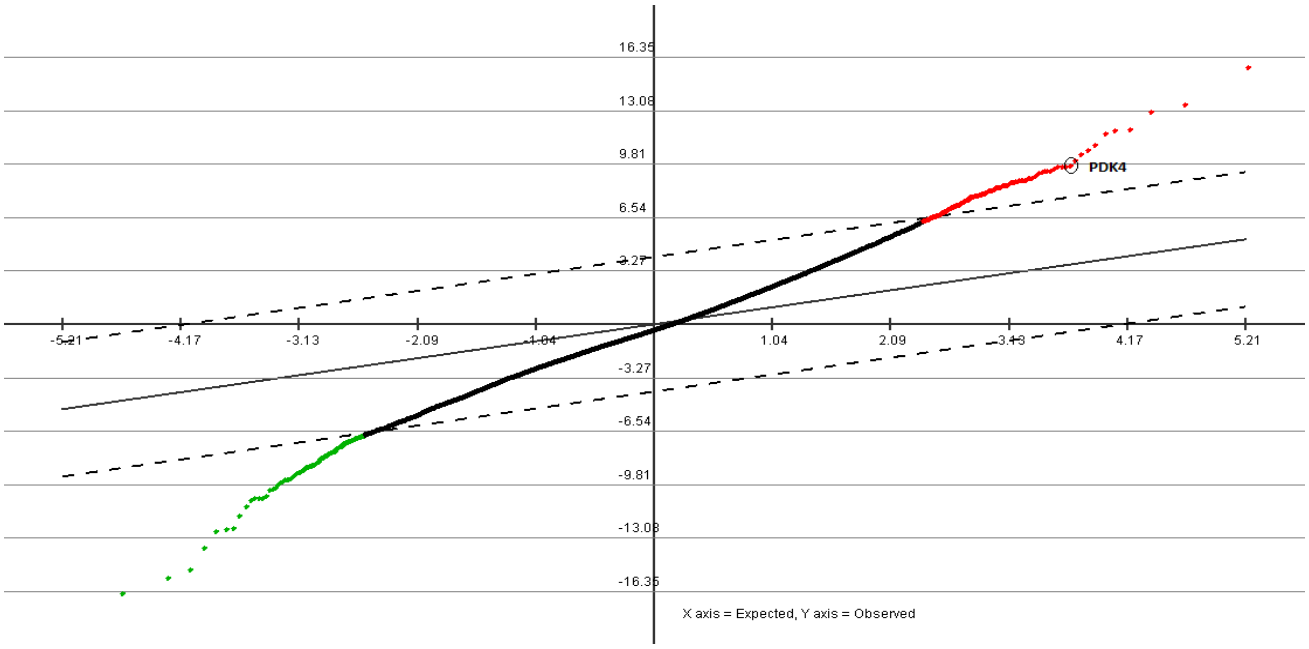

Figure 3. SAM graph of GSE6008 showing PDK4 expression level.

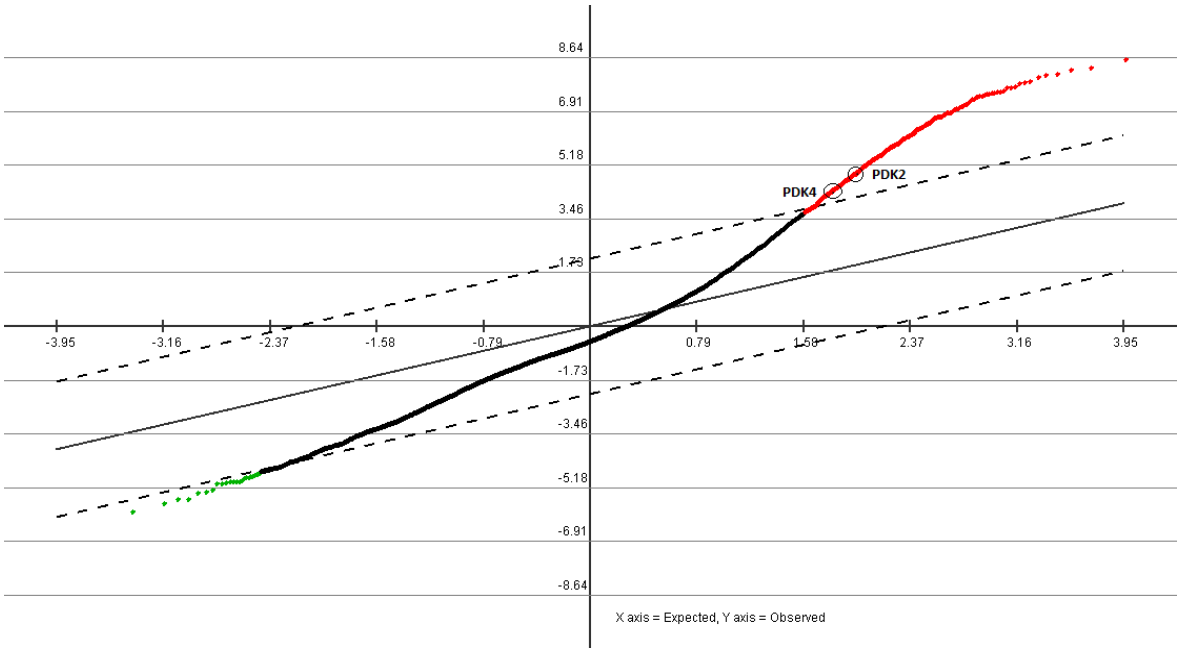

Figure 4. SAM graph of GSE4122 showing PDK4 and PDK2 over expression.

(Bowker-Kinley et al., 1998). As it was explained earlier that PDK's play a potential role in energy metabolism of the cell and any mutation in them can cause cancer. To date, the role of PDK's is unassigned in ovarian cancer. The PDK's major role is in phosphorylation which in turn makes them more important in cases of diabetes (Jeoung and Harris, 2010) and starvation. Naturally in case of starvation or fatty diet, PDK's are up regulated and this up regulation shuts down the PDK activity, which in turn preserves the pyruvate, lactate, and alanine. These carbon compounds are involved in glucose production and hence their conservation reduces the deposition of fats. This preservation of carbon compounds is very crucial for the survival in critical conditions of starvation and this job is done by PDK's. Now, how this phenomenon of PDK activity interacts in case of cancer is

Table 1. Showing p-values and fold change of PDK2 & PDK4 in both datasets.

Ref. ID	Gene	GSE4122		GSE6008	
		P value	Fold change	P value	Fold change
202590_s_at	PDK2	2.47E-05	1.08E+31	0.006889	1.1428 (non significant)
205960_at	PDK4	0.005181	4.16E+15	4.58E-13	2.064555

Table 2. SAGE results for PDK2 and PDK4.

Gene	Unigene cluster	SAGE Tag	Normal (tpm)		Ovarian cancer (tpm)	
			Tissues	Cell lines	Tissues	Cell lines
PDK2	Hs.256667	TGTGCTCAGG	No data	0	6	6
PDK4	Hs.8364	TAAATACTTG	No data	0	0	4

Table 3. Common microRNA's predicted by TargetScan, Pictar, miRanda and miRGen along with their binding energies.

	MicroRNA's	Binding energy (kcal/mol)
PDK4	hsa-miR-497	-116.9
	hsa-miR-424	-79.7
	hsa-miR-15b	-58.5
	hsa-miR-15a	-62.9
PDK2	hsa-miR-195	-85.6
	hsa-miR-16	-64.5
	hsa-miR-326	-94.9
	hsa-miR-330-5p	-27.7
	hsa-miR-1224-5p	-29.2
	hsa-miR-1208	-74.3

a very complicated question. While answering this query, we realized that the biological functioning of PDK's was never fully understood. The analysis of ovarian cancer expression data showed the significant over expression of PDK2 and PDK4, which disclosed their activity in cancer. As tumors have high energy demands and so in order to fulfil this requirement there is need of continuous glucose production and for this, the conversion of carbon compounds in fats should be blocked. This job can be performed by down regulating the PDC activity by the help of advanced regulation of PDK4 and PDK2.

Our results verify their over expression in ovarian cancer but normally there is no or very little expression of these PDK's. So, the enhanced level of expression in cancer supports our assumption about the process for the fulfilment of high energy demands of tumor cells. Another hypothesis for the up regulation of these PDKs in cancer is that they are involved in the enhancement of the resistance mechanism of tumors and make them able to withstand the hypoxic conditions produced as a result of greater glucose uptake of tumors. So for tumor growth and development, their expression level must be enhanced, apart from their normal behaviour. The role of PDK4 is a little strange, as it shows down regulation in tissues while performing SAGE and also in GENT one data set shows down regulation but at the same time, it was noticed that this down regulation is very minimal so it could be neglected. The reason for this might be that PDK4 is only over expressed in cell lines. The data set which shows little down regulation might be the data taken from tissues only. As there are strong evidences of over expression of PDK4 in ovarian cancer, so we ignored this minimal down expression.

Secondly, we also believed that the PDK4 is only over expressed in cell lines because of tumor growth and development of the cell line genes are mostly activated. Further investigation about difference in role of PDK4 in tissues and cell lines should be done in order to fully understand its working in cancer. The microRNA's can be best described as a regulator of their targeted genes. This process of regulation is at posttranscriptional level of the gene. They bind to the complementary sites present on the genes to which they target and hence block the translation and results in truncated products. The discovery of microRNA's forced the cancer scientists to think that only examining the expression of genes in cancer state is not sufficient but along with it, it is necessary to explore those genes which are actually regulating their expression. Hence the microRNA's are behind the scene players of the story of gene expression and without their presence the whole phenomena of gene expression can never be well understood. Here in this paper, after finding the PDK2 and PDK4 role in ovarian cancer, we also predicted their potential targeting microRNA's by different reliable and robust prediction algorithms. The increased differential expression of PDK2 and PDK4 in ovarian cancer reveals that their targeting microRNA's expression must be lowered during cancer state and therefore the over expression of these genes is possible, because if the microRNA's responsible for the control of PDK2 and PDK4 expression becomes higher in percentage than usual, then their blocking ability will be

enhanced, which will result in little or no expression of these genes in ovarian cancer.

The microarray analysis of these genes strongly convinced us about their over enhanced role in ovarian cancer, so we keep on thinking that all of the microRNA's for these genes in case of ovarian cancer must be down regulated. Recent studies also described that in case of cancers the microRNA's mostly happen to be down regulated (Meng et al., 2006; Lu et al., 2005). Some of the predicted microRNA's were never investigated before for their involvement in ovarian cancer. Scientists always linked the ovarian cancer with the breast cancer on the basis of their almost similar genetics (Bergfeldt et al., 2002). We predicted hsa-miR-497 as regulator of PDK4 in ovarian cancer. In a very recent study of February 2011, Li et al. reported the down regulation of has-miR-497 in breast cancer (Li et al., 2011). Similarly, the expression of hsa-miR-326 was also reported in breast cancer (Liang et al., 2010). These studies made these microRNA's as a strong candidate for their involvement in ovarian cancer. hsa-miR-424, hsa-miR-195 and hsa-miR-15a were already reported to be down regulated in case of ovarian cancer (Dahiya et al., 2008; Zhang et al., 2008). While the role of other predicted microRNA's has-miR-1208, hsa-miR-330-5p, hsa-miR-1224-5p is ambiguous, but future research on these microRNA's will untangle many interesting features as potential biological markers of ovarian cancer.

The present study is first to explore the role of energy metabolism in ovarian cancer. PDK role in metabolic reactions is very crucial. Although the precise role of PDKs remain unclear, we found the up regulation of PDK2, PDK4 and down regulation of their corresponding microRNA's in ovarian cancer. We believe that further *in vitro* investigations and more studies can be involved to verify the role of PDK in ovarian cancer. It will establish new dimensions for cancer research and therapeutics.

REFERENCES

Ao A, Wang H, Kamarajugadda S, Lu J (2008). Involvement of estrogen-related receptors in transcriptional response to hypoxia and growth of solid tumors. Proc. Nat. Acad. Sci. USA, 105: 7821-7826.

Araki M, Motojima K (2006). Identification of ERR alpha as a specific partner of PGC-1alpha for the activation of PDK4 gene expression in muscle. FEBS J., 273: 1669-1680.

Bergfeldt K, Rydh B, Granath F, Grönberg H, Thalib L, Adami H-O, Hall P (2002). Risk of ovarian cancer in breast-cancer patients with a familhistory of breast or ovarian cancer: a population-based cohort study. The lancet, pp. 891-894.

Betel D, Wilson M, Gabow A, Marks DS, Sander C (2008). The microRNA.org resource: targets and expression, Nucleic Acids Res., 36: 149-53.

Betel D, Koppal A, Agius P, Sander C and Leslie C (2010). Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. Genome Biol., 11: R90.

Boon K, Osorio EC, Greenhut SF, Schaefer CF, Shoemaker J, Polyak K, Morin PJ, Buetow KH, Strausberg RL, De Souza SJ, Riggins GJ (2002). An anatomy of normal and malignant gene expression. Proc. Nat. Acad. Sci. USA, 99: 11287-11292.

Bowker-Kinley MM, Davis WI, Wu P, Harris RA, Popov KM (1998). Evidence for existence of tissue-specific regulation of the mammalian pyruvate dehydrogenase complex. Biochem. J., 329: 191-196.

Carlson MW, Iyer VR, Marcotte EM (2007). Quantitative gene expression assessment identifies appropriate cell line models for individual cervical cancer pathways. BMC Genomics, 8: 117.

Cavalli LR, Liang BC (1998). Mutagenesis, tumorigenicity, and apoptosis: are the mitochondria involved? Mutation Research, 398: 19-26.

Chen Y, Cairns R, Papandreou I, Koong A, Denko NC (2009). Oxygen consumption can regulate the growth of tumors, a new perspective on the warburg effect. PLoS One, 4: e7033.

Dahiya N, Sherman-Baust CA., Wang T-L, Davidson B, Shih I-Y, Zhang Y, Wood W, Becker KG, Morin PJ (2008). MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One, 3: e2436.

Enright AJ, John B, Gaul U, Tuschl T, Sander C and Marks DS (2003). MicroRNA targets in Drosophila. Genome Biology, 5, R1.

Friedman RC, Farh KK, Burge CB, Bartel DP (2009). Most Mammalian mRNAs Are Conserved Targets of MicroRNAs. Genome Res., 19: 92-105.

Gordan JD, Simon MC (2007). Hypoxia-inducible factors: central regulators of the tumor phenotype. Curr. Opin. Genet. Div., 17: 71-77.

Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP (2007). "MicroRNA Targeting Specificity in Mammals: Determinants beyond seed pairing", Mol. Cell, 27: 91-105.

Grün D, Wang Y-L, Langenberger D, Gunsalus KC, Rajewsky N (2005). MicroRNA Target Predictions across Seven Drosophila Species and Comparison to Mammalian Targets. PLoS Comput. Biol., 1: e13.

Gudi R, Bowker-Kinley MM, Kedishvili NY, Zhao Y, Popov KM (1995). Diversity of the Pyruvate Dehydrogenase Kinase Gene Family in Humans. J. Biol. Chem., 270: 28989-28994.

Guerre-Millo M, Gervois P, Raspé E, Madsen L, Poulain P, Derudas B, Herbert J-M, Winegar DA, Willson TM, Fruchart J-C, Berge RK, Staels B (2000). Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. J. Biol. Chem., 275: 16638-16642.

Harris AL (2002). Hypoxia—A key regulatory factor in tumour growth. Nat. Rev. Cancer, 2: 38-47.

Jeoung NH, Harris RA (2010). Role of Pyruvate Dehydrogenase Kinase 4 in Regulation of Blood Glucose Levels. Korean Diabetes J., 34(5): 274-283.

John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS (2005). Human MicroRNA targets, PLoS Biol., 7: e264.

Kevin C, Nikolaus R (2006). Natural selection on human microRNA binding sites inferred from SNP data. Nat. Genet., 38: 1452-1456.

Kim JW, Tchernyshyov I, Semenza GL, Dang CV (2006). HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. Cell Metabol., 3: 177-185.

Krek A, Grün D, Poy MN. , Wolf R, Rosenberg L, Epstein EJ, MacMenamin P, Piedade ID, Gunsalus KC, Stoffel M, Rajewsky N (2005). "Combinatorial microRNA target predictions." Nat. Genet., 37(5):495-500.

Krüger J, Rehmsmeier M (2006). RNAhybrid: microRNA target prediction easy, fast and flexible. Nucl. Acids. Res., 34: W451-454.

Lall S, Grün D , Krek A, Chen K, Wang Y-L, Dewey CN, Sood P, Colombo T, Bray N, MacMenamin P, Kao H-L, Gunsalus KC, Pachter L, Piano F, Rajewsky N (2006). A Genome-Wide Map of Conserved MicroRNA Targets in *C. elegans*. Curr. Biol., 16: 460-471.

Lewis BP, Burge CB, Bartel DP (2005). "Conserved Seed Pairing, Often Flanked by Adenosines, Indicates that Thousands of Human Genes are MicroRNA targets". Cell, 120: 15-20.

Li D, Zhao Y, Liu C, Chen X, Qi Y, Jiang Y, Zou C, Zhang X, Liu S, Wang X, Zhao D, Sun Q, Zeng Z, Dress A, Lin MC., Kung H-F, Rui H, Liu L-Z, Mao F, Jiang B-H, Lai L (2011). Analysis of mir-195 and mir-497 expression, regulation and role in breast cancer. Published online first february 24: doi: 10.1158/1078-0432.ccr-10-1800.

Liang Z, Wu H, Xia J, Li Y, Yawei Zhang Y, Huang K, Wagar N, Yoon Y, Cho HT., Scala S, Shim H (2010). Involvement of miR-326 in chemotherapy resistance of breast cancer through modulating expression of multidrug resistance-associated protein 1. Biochem. Pharm., 79: 817-824.

Lin HJ, Hsieh F-C, Song H, Lin J (2005). Levated phosphorylation and activation of PDK-1/AKT pathway in human breast cancer. Br. J. Cancer, 93: 1372-1381.

Lu J, Getz G, Miska EA, Alvarez-saavedra E, Lamb J, Peck D, Sweet-cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR (2005). Microrna expression profiles classify human cancers. Nature, 435(7043): 834-838.

Majer M, Popov KM, Harris RA, Bogardus C, Prochazka M (1998). Insulin downregulates pyruvate dehydrogenase kinase (pdk) mrna: potential mechanism contributing to increased lipid oxidation in insulin-resistant subjects. Mol. Genet. Metab., 65: 181-186.

Maurer M, Su T, Saal LH, Koujak S, Hopkins BD, Barkley CR, Wu J, Nandula S, Dutta B, Xie Y (2009). 3- phosphoinositide-dependent kinase 1 potentiates upstream lesions on the phosphatidylinositol 3-kinase pathway in breast carcinoma. Cancer Res., 69: 6299-6306.

Mayevsky A (2009). mitochondrial function and energy metabolism in cancer cells: past overview and future perspectives. Mitochondrion, 9: 165-179.

Megraw M, Sethupathy P, Benoit Corda B, Hatzigeorgiou AG (2006). Mirgen: A database for the study of animal microrna genomic organization and function. Nucleic Acids Res., 35: d149-d155.

Meng F, Henson R, Lang M, Wehbe H, Maheshwari S, Mendell JT, Jiang J, Schmittgen TD, Patel T (2006). Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. Gastroenterology, 130: 2113-2129.

Ramanathan A, Wang C, Stuart L, Schreiber SL (2005). Perturbational profiling of a cell-line model of tumorigenesis by using metabolic measurements. Proc. Nat. Acad. Sci., 102: 5992-5997.

Rehmsmeier M, Steffen P, Hochsmann M, Giegerich R (2004). Fast and effective prediction of microRNA/target duplexes RNA. 10: 1507-1517.

Semenza GL (2003) Targeting HIF-1 for cancer therapy. Nat. Rev. Cancer, 3: 721-732.

Seyfried TN, Mukherjee P (2005) Targeting energy metabolism in brain cancer: review and hypothesis. Nutr. Metab. (Lond), 2: 30.

Seyfried TN, Shelton LM. (2007) Cancer as a Metabolic Disease. Nutr. Metab., 7: 7.

Shin G, Kang WT, Yang S, Baek SJ, Jeong YS, Kim SY (2011). “GENT: Gene Expression Database of Normal and Tumor Tissues”. Cancer Inf., 10: 149-157.

Stine KE (1956). On-line review: Energy metabolism and cancer.

Warburg O (1956). On the origin of cancer cells. Science, 123: 309-314.

Wende AR, Huss JM, Schaeffer PJ, Giguère V, Kelly DP (2005) PGC-1alpha coactivates PDK4 gene expression via the orphan nuclear receptor ERRalpha: a mechanism for transcriptional control of muscle glucose metabolism. Mol. Cell Biol., 25: 10684-10694.

Wu P, Inskeep K, Bowker-Kinley MM, Popov KM, Harris RA (1999). Mechanism responsible for inactivation of skeletal muscle pyruvate dehydrogenase complex in starvation and diabetes, 48(8): 1593-1599.

Zhang L, Volinia S, Bonome T, Calin GA, Greshock J, Yang N, Liu C-G, Giannakakis A, Alexiou P, Hasegawa K, Johnstone CN, Megraw MS, Adams S, Lassus H, Huang J, Kaur S, Liang S, Sethupathy P, Leminen A, Simossis VA, Sandaltzopoulos R, Naomoto Y, Katsaros D, Gimotty PA, DeMichele A, Huang O, Ralf Bützow R, Rustgi AK, Weber BL, Birrer MJ, Hatzigeorgiou AG, Croce CM, Coukos G (2008). Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer. PNAS, 105: 7004 –7009

Zhang Y, Ma K, Sadana P, Chowdhury F, Gaillard S, Wang F, McDonnell DP, Unterman TG, Elam MB, Park EA (2006). Estrogen-related receptors stimulate pyruvate dehydrogenase kinase isoform 4 gene expression. J. Biol. Chem., 281: 39897-39906.