MicroRNA Expression Analysis Reveals Significant Biological Pathways in Human Prostate Cancer

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***Abstract*—MicroRNAs (miRNAs) are reported to play essential roles in cancer initiation and progression and microarray technologies are intensively applied to study the miRNA expression profile in cancer. It is very common that the set of differentially expressed miRNAs related to the same cancer identified from different laboratories varies widely. Meanwhile, how the altered miRNAs coordinately contribute to the cause of prostate cancer is still not clear. In this study, we collected and processed four human prostate cancer associated miRNA microarray expression datasets with newly developed cancer outlier detection methods to identify differentially expressed miRNAs (DE-miRNAs). The targets of these DE-miRNAs were then extracted from database or predicted by bioinformatics prediction and then mapped to functional databases for enrichment analysis and overlapping comparison. Newly developed outlier detection methods were found to be more appropriate than t-test in cancer research, and the consistency of independent prostate cancer expression profiles at pathway or gene-set level was shown higher than that at gene (*i.e.* miRNA here) level. Furthermore, we identified 41 Gene Ontology terms, 4 KEGG pathways and 77 GeneGO pathways which are associated with prostate cancer. Among the top 15 GeneGO pathways, 5 were reported previously and the rest could be putative ones. Our analyses showed that more appropriate outlier detection methods should be used to detect oncogenes or oncomiRNAs that are altered only in a subset of samples. We proved that expression signatures of independent microarray experiments are more consistent rather at pathway level than at miRNA / gene level. We also found that the utilization of similar meta-analysis methods between miRNA and mRNA profiling datasets result in the detection of the same pathways.**

Keywords-Meta-analysis; microRNA expression; pathway enrichment; GeneGO's database

I. INTRODUCTION

MicroRNAs (miRNAs) are approximately 22-nucleotide endogenous RNAs that play important gene-regulatory roles in animals and plants. They target the 3' Untranslated Region (3'UTR) of mRNAs to repress the activity of complementary mRNAs [1]. According to the latest miRBase [2] release, more than 1000 mature miRNAs have been identified in the human genome. Abnormalities of miRNA expression might contribute to the generation of "cancer stem cell", which may eventually transform to tumors [3-5].

Microarray technologies have become routine methods for profiling molecular expression in almost all fields of research in life sciences. In the field of cancer research, microarray technologies allow simultaneous assessment of transcription of tens of thousands of genes, and comparison of relative expressions between normal and malignant cells. With more and more published microarray expression datasets available, it appears to be possible and also necessary to apply meta-analysis methods to find significant patterns from multiple datasets. Efforts have been made to identify common gene signatures. However, it is very common that the set of differentially expressed miRNAs (DE-miRNAs) reported from different laboratories varies widely, although they are related to the same cancer [6-11].

In this study, we collected and processed 4 miRNA expression microarray datasets by meta-analysis method at both gene and gene-set (i.e. the functional gene set or pathway) levels to prove our hypothesis that the expression signatures of independent datasets are more consistent at pathway level than at miRNA / gene level. Based on this hypothesis, we also identified novel prostate cancer associated pathways targeted by miRNAs coordinately.

II. RESULTS

A. Newly developed outlier detection algorithms perform better than t-test

In most of the previous work, researchers often apply fold change or t-test / t-test like statistics to detect cancer related genes and miRNAs after obtaining microarray expression profiles. Recently, it has been recognized that many genes show a differential expression only in a small proportion of normal and cancer samples [12] . The patterns hidden in the subsets of the samples cannot be discovered with t-test like methods since with these methods, the gene expression patterns will be averaged out. Tomlins and others proved that t-test was hardly useful in such cases [13].

To address this complexity, a series of new outlier detection algorithms were proposed in recent years. Among these methods, there are Least Sum of Ordered Subset Square t-statistic-LSOSS [14], Cancer Outlier Profile Analysis-COPA [15], Maximum Ordered Subset T-statistics-MOST [12], Outlier Robust T-statistics-ORT [16], and Outlier Sum-OS [17]. We compared the performance of these newly developed algorithms with t-test in outlier detection. For all these methods, we set the quantile of outliers to 0.05 (5%). After obtaining the outliers of each dataset by various methods, those differentially expressed miRNAs (DE-miRNAs) shared by the results of at least 3

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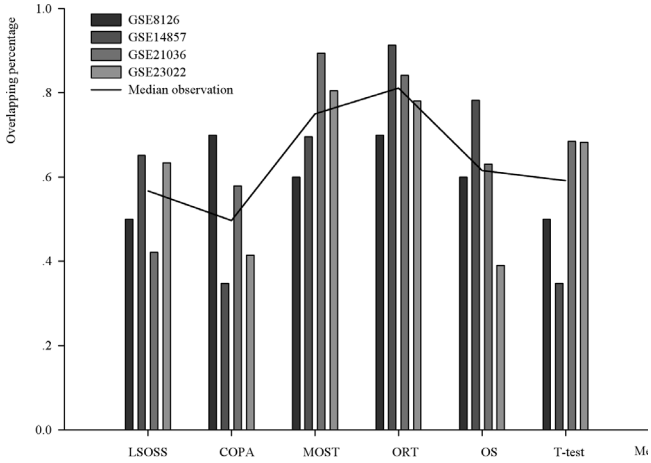

Standard deviation	0.1105	0.1594	0.1283	0.0906	0.1616	0.1623

Figure 1. **Overlapping percentages of putative outliers in the original ones by the newly developed and traditional outlier detection methods.** Outliers shared by at least 3 methods were considered as putative ones.

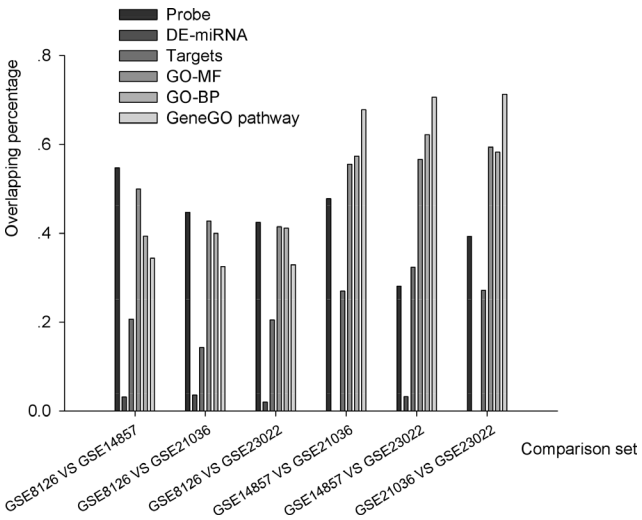

Figure 2. **Pair-wise comparison between 4 datasets at different levels.** X axis shows the total 6 pair-wise comparison sets for 4 datasets, while Y axis denotes the overlapping percentage in a decimal format. Bars in different gray intensity stand for the overlapping at different levels, including probe, DE-miRNA, targets, GO-MF, GO-BP, and GeneGO pathway.

methods were considered as putative ones, and then the percentage of these putative outliers in the original result of each method was calculated as the accuracy (See Fig. 1). In Fig. 1, all the new methods show smaller standard deviations than that of t-test, which indicates that these algorithms have more stable performance when dealing with these different expression datasets. Among of them, ORT performed best for expression outlier extraction from these four datasets, and all of the downstream analysis was based on the result of ORT.

B. Target genes of DE-miRNAs were obtained and mapped to functional databases

Since miRNAs play an important role of posttranscriptional regression by targeting mRNAs, we studied the function of the DE-miRNAs by identifying putative targets and subsequently mapping these targets to available functional databases. Target genes were retrieved from both TargetScan database and result of local prediction process. (See methods section for detailed information). Additional file 2 shows the target genes of DE-miRNAs for each dataset. 1085 unique targets for DE-miRNA of GSE8126, 2928 for GSE14857, 2678 for GSE21036 and 3052 for GSE23022 were obtained, respectively. Afterwards, these targets from each individual dataset were mapped to several functional databases including GO [18] , KEGG [19, 20] and GeneGo's database (GeneGo, Inc.).

C. Higher consistency can be reached at functional level than that at miRNA / target level

We performed overlapping analysis across the four datasets at different levels with the mapping data above. Fig. 2 shows the pair-wise comparison between four datasets at the level of probe, DE-miRNA, targets, GO-MF (Molecular Function), GO-BP (Biological Process) and GeneGO pathway, respectively. Then we calculated the p-value by paired t-test to show the statistics of the overlapping percentages difference at different levels (see Table I). We compared overlapping percentage at GO-MF, GO-BP and GeneGO pathway level with that of probe, DE-miRNA and target. According to the statistics shown in Table I, it is clear that there is no significant difference between the overlapping percentages at GO-MF / GO-BP / GeneGO pathway level and at probe level, while the difference between the overlapping percentages at GO-MF / GO-BP / GeneGO level and at DE-miRNA / target level are statistically significant. Our results demonstrate that the expression signatures of independent datasets are more consistent at GO or pathway level than at miRNA / target level.

D. Identifying prostate cancer related pathways

In order to identify prostate cancer associated GO terms and pathways, we first extracted those significant target genes shared by at least 3 datasets, which we then mapped to GO, KEGG pathway and GeneGO pathway databases for the functional enrichment analysis. In this process, we extracted 1158 target genes, among which 275 were shared by 4 datasets and 883 were overlapped in 3 datasets. Fig. 3 shows the most significant GeneGO pathway, TGF, WNT and

TABLE I. Statistics of comparison of overlapping percentages at different levels.

Levels		Paired t-test (*p*-value)
GeneGO pathway	***Probe***	0.451
	DE-miRNA	2.23E-03
	Target	5.42E-03
GO-MF	***Probe***	0.201
	DE-miRNA	3.24E-05
	Target	1.39E-05
GO-BP	***Probe***	0.389
	DE-miRNA	1.60E-04
	Target	6.92E-05

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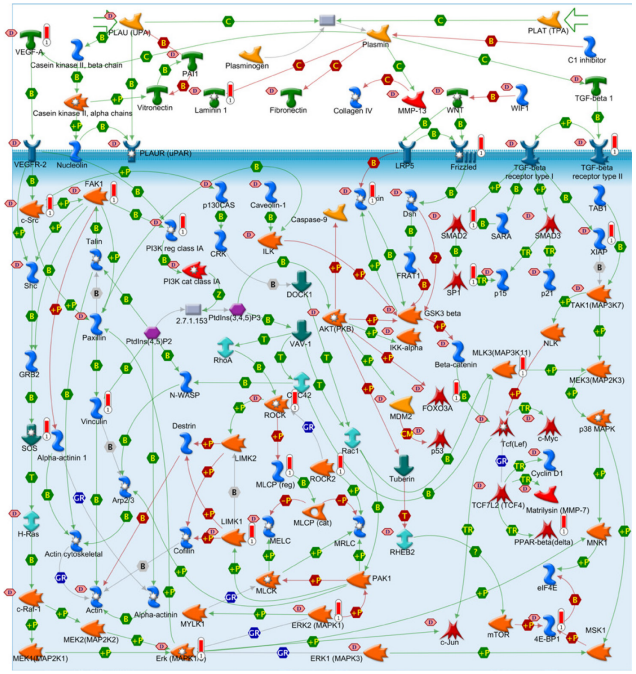

Figure 3. **The most significant GeneGO pathway map of Cytoskeleton remodeling_TGF, WNT and cytoskeletal remodeling.** Additional file 1 shows the legend for this map. The target genes of the putative DE-miRNA are represented with red bar histograms. The little hexagon in the color of light red labeled with "D" means that this object is associated with the Prostate cancer.

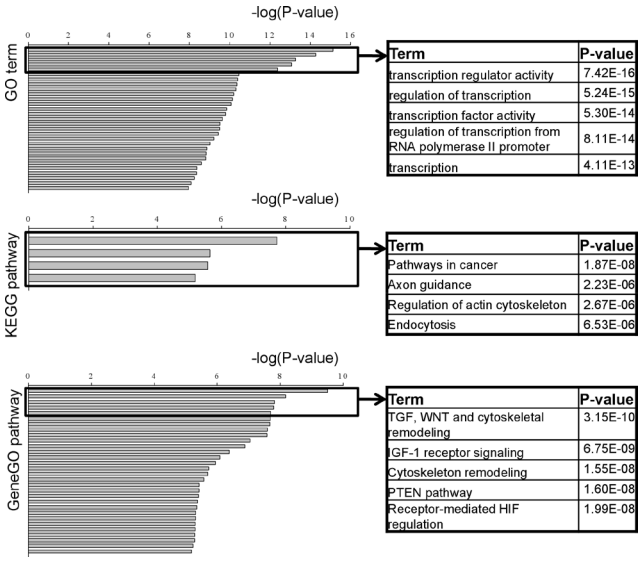

Figure 4. **Illustration of various biological theme enrichment.** DE-miRNAs shared by at least 3 datasets were extracted to identify target genes and these genes were then mapped to databases for the identification of enriched GO terms (FDR < 0.001), KEGG pathways (FDR < 0.01). Top GO terms, KEGG and GeneGO pathways were shown in this illustration. Terms shown in the box to the right of each bar plot are the most significant ones.

cytoskeletal remodeling, see additional file 1 for the notation of each sign in this figure. In Fig. 3, TGF-beta 1 encodes a member of TGFB family, which regulates important functions such as proliferation, differentiation, migration and others in many kinds of cells. TGF-beta 1 is supposed to binds TGF-beta receptor type II and transfer the signal to type I and activates SMAD2 directly or via binding SARA, then encodes a double zinc finger protein that interacts with SMAD2, and finally SMAD2 will bind to SP1. Both SMAD2 and SP1 are transcription factors found to be important during the formation and development of prostate tumors. TGF-beta receptor type II, SMAD2 and SP1 are all target genes of the identified DE-miRNAs in our analysis. Fig. 4 illustrates various biological theme enrichment for the gene list. The left part of the figure is the bar plot of enriched GO terms, KEGG pathways and GeneGO pathways against –log10 (p-value); the right part shows top 5 terms of each biological theme.

Detailed information is also available in additional file 3, 4 and 5. In these files, terms were sorted by p-value. Overall, we identified 41 (FDR < 0.001) GO terms, 4 (FDR < 0.01) KEGG pathways and 77 (FDR < 0.001) GeneGO pathways, which are enriched with target genes of prostate cancer associated DE-miRNAs.

E. Validation of the result

Among the 77 enriched GeneGO pathways, 50 (64.9%) pathways were also found to be significantly enriched in our previous study in which we processed 10 mRNA microarray datasets [11]. In the set of top 15 GeneGO pathways in our previous work, 9 (60%) were shared in this study (See additional file 5 for detail).

To identify potential prostate cancer related pathways, 15 most significantly enriched (with lowest p-value) pathways were picked out. 5 of them were found to be related to prostate cancer according to text-mining searches in PubMed, and the rest could be potential prostate cancer related pathways which need more wet-lab experiments to validate. (Table II) Citation counts of corresponding genes in each potential pathway could be found in additional file 6. Percentage of prostate cancer related genes in each pathway according to PubMed text-mining ranges from 33.33% to 83.33%.

III. DISCUSSION

In this study, we collected four miRNA microarray datasets with the samples of normal prostate tissue and prostate cancer tissue, and processed these datasets in a meta-analysis method to identify the signature at both miRNA / target gene and pathway levels.

As it has been recognized recently that individual variation, especially, the alteration of oncogenes or oncomiRNAs may occur in a subset of cancer samples, should be considered in microarray experiments, the present work is the first article comparing the performance of different outlier detection methods with real datasets. We performed outlier detection by both traditional method (t-test) and newly developed approaches (LSOSS, COPA, MOST, ORT, OS). The accuracy analysis draws a conclusion that newly developed algorithms generally perform better than t-test. The variation of performance for different datasets may be a result of different sizes of the datasets and with the small number of datasets, the advantage against t-test is not apparent for the new methods. Compared with traditional t-test or t-test like methods, newly developed methods are generally more appropriate for

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TABLE II. Top 15 enriched GeneGO pathways with PubMed citation count.

Category	Term	PubMed citation count *
Cytoskeleton remodeling	TGF, WNT and cytoskeletal remodeling	875
Development	IGF-1 receptor signaling	
Cytoskeleton remodeling	Cytoskeleton remodeling	10
Signal transduction	PTEN pathway	525
Transcription	Receptor-mediated HIF regulation	
Muscle contraction	Regulation of eNOS activity in endothelial cells	
Development	PIP3 signaling in cardiac myocytes	
Cardiac Hypertrophy	NF-AT signaling in Cardiac Hypertrophy	
Cytoskeleton remodeling	Role of Activin A in cytoskeleton remodeling	
Transcription	CREB pathway	2
Development	Role of HDAC and calcium/calmodulin-dependent kinase (CaMK) in control of skeletal myogenesis	
Development	GM-CSF signaling	
Development	Flt3 signaling	
Transcription	Role of heterochromatin protein 1 (HP1) family in transcriptional silencing	
Cytoskeleton remodeling	FAK signaling	3

* Citation count was calculated by searching PubMed in the fields of title and abstract, and this may change with the update of PubMed.

microarray data analysis in cancer study, esp. for large datasets.

The result of consistency analysis at different levels is accordant with our hypothesis that the expression signatures of independent datasets are more consistent at pathway level than at gene level. This also partially explains why independent microarray experiments result in quite different outliers. As we all know, cancer is a complex disease caused by various environmental and genetic factors, and different causes may result in different alteration of biological function and symptoms. As for prostate cancer, different mechanisms attribute to cancer formation in various parts of the world such as different dietary habits and environment. However, there should be some common key pathways playing important roles in the process of cancer formation.

Fig. 4 illustrates the GO terms or pathways (both from KEGG and GeneGO) that are enriched by the list of common genes from four datasets. According to the figure, top 5 enriched GO terms are all related to transcription and its regulation, which may be explained by the abnormal gene expression in prostate tumors. The identified significant KEGG and GeneGO pathways are mainly important pathways in cancer, intercellular communication and cytoskeleton remodeling, all of which are usually involved in the process of transformation of cell shape or metastasis of tumor. A recent review [21] suggested that Wnt signaling regulates androgen activity in prostate cancer cells, enhances androgen receptor expression and promotes the growth of prostate tumor. As stated by Eric Chu, "PTEN is the first phosphatase identified as a tumor suppressor". PTEN (phosphatase and tensin homolog) plays a key role in apoptosis, cell cycle arrest, and possibly cell migration [22]. Evidence has also been accumulated showing that the Insulin-Like Growth Factor (IGF) family is involved in the regulation of prostate growth and bone metastasis [23]. All the evidences above corroborate the credibility of the results of the present study.

The combination of our previous study [11] with the present one shows the high consistency between results of similar meta-analysis for miRNA and mRNA microarray datasets measured from prostate tissues (normal and malignant). These overlapping pathways could be potential key pathways contributing to prostate carcinogenesis; in the foreseeable future, drugs designed to modify these pathways might be useful in interrupting the process of tumor formation. In addition, we identified 10 novel prostate cancer related pathways, see details in Table II. IGF-1 receptor is a tyrosine kinase receptor which is related to tumor progression and metastasis, as Sroka *etc.* [24] reported, in prostate cancer cells it is highly expressed with MT1-MMP, a metalloproteinase involved in prostate cancer metastasis. It has been shown that abnormalities of HIF expression mediate lethal processes such as cell survival, proliferation and angiogenesis [25, 26]. According to the work by Wartenberg *etc.* [27], the expression of endothelial nitric oxide synthase (eNOS) is down-regulated in spheroid prostate tumors and which contributes to tumorigenesis by mediating nitric oxide (NO). Activin A was found to inhibit prostatic branching and growth [28] and enhances prostate cancer cell migration [29]. In a

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paper published in 2009 [30], histone deacetylaces (HDACs) were detected with increased expression and therefore promote the prostate cancer cell growth and invasion. Additional file 6 shows the citation counts of corresponding genes of each potential pathway in prostate cancer. More experiments are needed to confirm whether these pathways are actually related to prostate cancer or not.

IV. CONCLUSIONS

In this study, by showing the performance of various outlier detection methods with four real microRNA microarray datasets, we demonstrated that new methods perform better than t-test in the microarray data analysis in cancer research, duo to the fact that differentially expressed genes can be only

identified in subsets of cancer samples. We also demonstrated that expression signatures of independent microarray experiments are more consistent at pathway level than at gene level, which can partially explain the inconsistency between results of independent microarray experiments at gene level. After comparing our results with previous work, we concluded that same pathways could be obtained by similar meta-analysis methods on miRNA and mRNA profiling datasets.

V. MATERIALS AND METHODS

A. Collecting datasets

The miRNA expression microarray datasets used in this study were retrieved from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/), a public functional genomics data repository supporting MIAME-compliant data submissions. These datasets were collected from four platforms with miRNA probes designed using Sanger miRBase release, and were downloaded in single matrix file format. (Table III) Considering the diverse platforms of the datasets, local Blast [31] was performed by mapping probe sequences to the miRNA precursors of latest miRBase (release 16 [2]) to identify the concordant miRNA names. Fig. 5 displays the pipeline of the whole procedure in this study.

B. Comparison of these outlier detection algorithms

In this study, expression outliers of these four datasets were determined with six methods which include LSOSS, COPA, MOST, ORT, OS, and t-test. All these methods were implemented in R packages by Wang [14] and Lian [12], which was used to perform the outlier detection. The quantile of outlier extraction for all the methods was set to 0.05 (5%) by default.

After the DE-miRNAs obtained by the six methods, we compared their performance first by collecting the DE-miRNAs shared by at least three methods, taking them as the putative outliers. Then the percentage of these DE-miRNAs in the original result of each method was calculated as the accuracy.

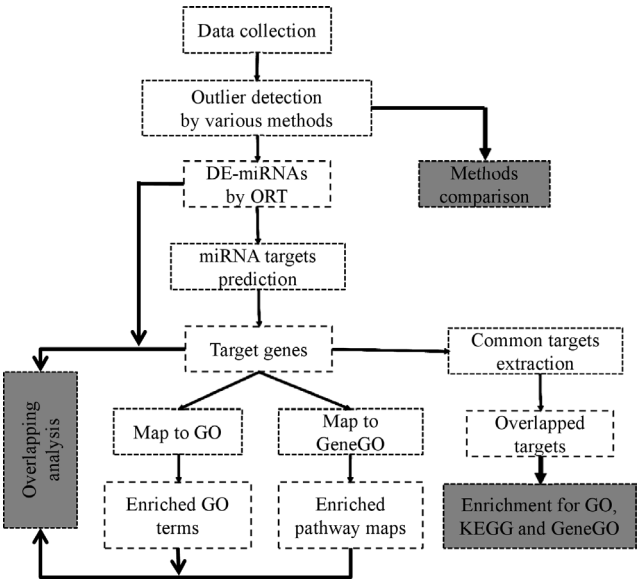

Figure 5. **The meta-analysis pipeline in this study.**

C. Determining the differentially expressed miRNAs

As Fig. 1 shows, we considered ORT as the most appropriate method for the detection of outliers. The outliers detected by ORT were used in the downstream analysis.

D. Obtaining target genes for differentially expressed miRNAs

TargetScan database was chosen to retrieve target genes of DE-miRNAs. A series of in-house Perl scripts were developed to obtain the targets of miRNAs available in the database; for those miRNAs unavailable in the TargetScan database, we manually predicted their putative target genes by performing a genome-wide, sequence-based bioinformatics procedure, using three of the most popular tools, miRanda [32], RNAhybrid [33], and TargetSpy [34]. These tools use both miRNA sequences and 3'UTR (3' Untranslated Region) of protein-coding mRNA sequences as input files generally in fasta format and determine their binding ability by calculating the minimum free energy for hybridization. Human 3'UTR data was downloaded from PACdb build36 [35]. After the prediction of putative miRNA target genes by each tool with the default parameters, overlapped target genes were extracted in order to obtain a more solid result.

E. Performing functional enrichment analysis

To study the function of those DE-miRNAs we mapped their targets to GO, KEGG and GeneGO databases. In the consistency analysis part, to eliminate the error caused by different enrichment tools, we mapped targets of each dataset to GO-MF, GO-BP, and GeneGO pathway databases by

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TABLE III. Summary of prostate tissue datasets used in this study

Dataset	GEO accession NO.	Platforms	Human miRNA probes	Number of samples		Statistics	Ref.
				Prostate normal tissue	*Prostate cancer tissue*		
1	GSE8126	Agilent-016436	474	16	60	T-test	[36]
2	GSE14857	Affymetrix miRNA Array	407	12	12	T-test	[37]
3	GSE21036	Agilent-019118	373	28	113	Mixture model	[38]
4	GSE23022	Affymetrix miRNA Array	847	20	20	ANOVA	[39]

MetaCore™; while for the identification of prostate cancer related pathways, we first picked out targets shared by at least 3 datasets, which were then mapped to GO, KEGG pathway database by DAVID and to GeneGO pathway database by MetaCore™. Both of DAVID and MetaCore™ used hypergeometric distribution to calculate the significance level (p-value) for each pathway and adjust it by FDR value, which was used as threshold. In MetaCore™, p-value means the probability of a random intersection of two different gene sets. The low p-value indicates the high potential of non-randomness of the finding.

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REFERENCES

[1] D.P. Bartel, "MicroRNAs: target recognition and regulatory functions," *Cell*, vol. 136, (no. 2), pp. 215-33, Jan 23 2009.

[2] A. Kozomara and S. Griffiths-Jones, "miRBase: integrating microRNA annotation and deep-sequencing data," *Nucleic Acids Res*, vol. 39, (no. Database issue), pp. D152-7, Jan 2010.

[3] S.L. Lin, D.C. Chang, S.Y. Ying, D. Leu, and D.T. Wu, "MicroRNA miR-302 inhibits the tumorigenecity of human pluripotent stem cells by coordinate suppression of the CDK2 and CDK4/6 cell cycle pathways," *Cancer Res*, vol. 70, (no. 22), pp. 9473-82, Nov 15 2010.

[4] C. Liu, K. Kelnar, B. Liu, X. Chen, T. Calhoun-Davis, H. Li, L. Patrawala, H. Yan, C. Jeter, S. Honorio, J.F. Wiggins, A.G. Bader, R. Fagin, D. Brown, and D.G. Tang, "The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44," *Nat Med*, vol. 17, (no. 2), pp. 211-5, Feb 2011.

[5] B. Mallick, J. Chakrabarti, and Z. Ghosh, "MicroRNA reins in embryonic and cancer stem cells," *RNA Biol*, vol. 8, (no. 3), May 1 2011.

[6] D.R. Rhodes, T.R. Barrette, M.A. Rubin, D. Ghosh, and A.M. Chinnaiyan, "Meta-analysis of microarrays: interstudy validation of gene expression profiles reveals pathway dysregulation in prostate cancer," *Cancer Res*, vol. 62, (no. 15), pp. 4427-33, Aug 1 2002.

[7] P. Cahan, F. Rovegno, D. Mooney, J.C. Newman, G. St Laurent, 3rd, and T.A. McCaffrey, "Meta-analysis of microarray results: challenges, opportunities, and recommendations for standardization," *Gene*, vol. 401, (no. 1-2), pp. 12-8, Oct 15 2007.

[8] L. Ein-Dor, I. Kela, G. Getz, D. Givol, and E. Domany, "Outcome signature genes in breast cancer: is there a unique set?," *Bioinformatics*, vol. 21, (no. 2), pp. 171-8, Jan 15 2005.

[9] S. Michiels, S. Koscielny, and C. Hill, "Prediction of cancer outcome with microarrays: a multiple random validation strategy," *Lancet*, vol. 365, (no. 9458), pp. 488-92, Feb 5-11 2005.

[10] J.E. Korkola, E. Blaveri, S. DeVries, D.H. Moore, 2nd, E.S. Hwang, Y.Y. Chen, A.L. Estep, K.L. Chew, R.H. Jensen, and F.M. Waldman, "Identification of a robust gene signature that predicts breast cancer outcome in independent data sets," *BMC Cancer*, vol. 7, pp. 61, 2007.

[11] Y. Wang, J. Chen, Q. Li, H. Wang, G. Liu, Q. Jing, and B. Shen, "Identifying novel prostate cancer associated pathways based on integrative microarray data analysis," *Computational Biology and Chemistry*, vol. In Press, Accepted Manuscript, 2011.

[12] H. Lian, "MOST: detecting cancer differential gene expression," *Biostatistics*, vol. 9, (no. 3), pp. 411-8, Jul 2008.

[13] S.A. Tomlins, D.R. Rhodes, S. Perner, S.M. Dhanasekaran, R. Mehra, X.W. Sun, S. Varambally, X. Cao, J. Tchinda, R. Kuefer, C. Lee, J.E. Montie, R.B. Shah, K.J. Pienta, M.A. Rubin, and A.M. Chinnaiyan, "Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer,"

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Science, vol. 310, (no. 5748), pp. 644-8, Oct 28 2005.

[14] Y. Wang and R. Rekaya, "LSOSS: Detection of Cancer Outlier Differential Gene Expression," *Biomark Insights*, vol. 5, pp. 69-78, 2010.

[15] J.W. MacDonald and D. Ghosh, "COPA--cancer outlier profile analysis," *Bioinformatics*, vol. 22, (no. 23), pp. 2950-1, Dec 1 2006.

[16] B. Wu, "Cancer outlier differential gene expression detection," *Biostatistics*, vol. 8, (no. 3), pp. 566-75, Jul 2007.

[17] R. Tibshirani and T. Hastie, "Outlier sums for differential gene expression analysis," *Biostatistics*, vol. 8, (no. 1), pp. 2-8, Jan 2007.

[18] M. Ashburner, C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J.M. Cherry, A.P. Davis, K. Dolinski, S.S. Dwight, J.T. Eppig, M.A. Harris, D.P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J.C. Matese, J.E. Richardson, M. Ringwald, G.M. Rubin, and G. Sherlock, "Gene ontology: tool for the unification of biology. The Gene Ontology Consortium," *Nat Genet*, vol. 25, (no. 1), pp. 25-9, May 2000.

[19] M. Kanehisa, M. Araki, S. Goto, M. Hattori, M. Hirakawa, M. Itoh, T. Katayama, S. Kawashima, S. Okuda, T. Tokimatsu, and Y. Yamanishi, "KEGG for linking genomes to life and the environment," *Nucleic Acids Res*, vol. 36, (no. Database issue), pp. D480-4, Jan 2008.

[20] M. Kanehisa, S. Goto, M. Furumichi, M. Tanabe, and M. Hirakawa, "KEGG for representation and analysis of molecular networks involving diseases and drugs," *Nucleic Acids Res*, vol. 38, (no. Database issue), pp. D355-60, Jan 2010.

[21] G. Kharaishvili, D. Simkova, E. Makharoblidze, K. Trtkova, Z. Kolar, and J. Bouchal, "Wnt signaling in prostate development and carcinogenesis," *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, vol. 155, (no. 1), pp. 11-8, Mar 2011.

[22] E.C. Chu and A.S. Tarnawski, "PTEN regulatory functions in tumor suppression and cell biology," *Med Sci Monit*, vol. 10, (no. 10), pp. RA235-41, Oct 2004.

[23] C. Gennigens, C. Menetrier-Caux, and J.P. Droz, "Insulin-Like Growth Factor (IGF) family and prostate cancer," *Crit Rev Oncol Hematol*, vol. 58, (no. 2), pp. 124-45, May 2006.

[24] I.C. Sroka, K. McDaniel, R.B. Nagle, and G.T. Bowden, "Differential localization of MT1-MMP in human prostate cancer tissue: role of IGF-1R in MT1-MMP expression," *Prostate*, vol. 68, (no. 5), pp. 463-76, Apr 1 2008.

[25] J. Mathieu, Z. Zhang, W. Zhou, A.J. Wang, J.M. Heddleston, C.M. Pinna, A. Hubaud, B. Stadler, M. Choi, M. Bar, M. Tewari, A. Liu, R. Vessella, R. Rostomily, D. Born, M. Horwitz, C. Ware, C.A. Blau, M.A. Cleary, J.N. Rich, and H. Ruohola-Baker, "HIF Induces Human Embryonic Stem Cell Markers in Cancer Cells," *Cancer Res*, vol. 71, (no. 13), pp. 4640-52, Jul 1 2011.

[26] J. Shin, H.J. Lee, D.B. Jung, J.H. Jung, E.O. Lee, S.G. Lee, B.S. Shim, S.H. Choi, S.G. Ko, K.S. Ahn, S.J. Jeong, and S.H. Kim, "Suppression of STAT3 and HIF-1 Alpha Mediates Anti-Angiogenic Activity of Betulinic Acid in Hypoxic PC-3 Prostate Cancer Cells," *PLoS One*, vol. 6, (no. 6), pp. e21492, 2011.

[27] M. Wartenberg, M. Schallenberg, J. Hescheler, and H. Sauer, "Reactive oxygen species-mediated regulation of eNOS and iNOS expression in multicellular prostate tumor spheroids," *Int J Cancer*, vol. 104, (no. 3), pp. 274-82, Apr 10 2003.

[28] B. Cancilla, R.A. Jarred, H. Wang, S.L. Mellor, G.R. Cunha, and G.P. Risbridger, "Regulation of prostate branching morphogenesis by activin A and follistatin," *Dev Biol*, vol. 237, (no. 1), pp. 145-58, Sep 1 2001.

[29] H.Y. Kang, H.Y. Huang, C.Y. Hsieh, C.F. Li, C.R. Shyr, M.Y. Tsai, C. Chang, Y.C. Chuang, and K.E. Huang, "Activin A enhances prostate cancer cell migration through activation of androgen receptor and is overexpressed in metastatic prostate cancer," *J Bone Miner Res*, vol. 24, (no. 7), pp. 1180-93, Jul 2009.

[30] L. Wang, X. Zou, A.D. Berger, C. Twiss, Y. Peng, Y. Li, J. Chiu, H. Guo, J. Satagopan, A. Wilton, W. Gerald, R. Basch, Z. Wang, I. Osman, and P. Lee, "Increased expression of histone deacetylaces (HDACs) and inhibition of prostate cancer growth and invasion by HDAC inhibitor SAHA," *Am J Transl Res*, vol. 1, (no. 1), pp. 62-71, 2009.

[31] C. Camacho, G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, and T.L. Madden, "BLAST+: architecture and applications," *BMC Bioinformatics*, vol. 10, pp. 421, 2009.

[32] B. John, A.J. Enright, A. Aravin, T. Tuschl, C. Sander, and D.S. Marks, "Human MicroRNA targets," *PLoS Biol*, vol. 2, (no. 11), pp. e363, Nov 2004.

[33] M. Rehmsmeier, P. Steffen, M. Hochsmann, and R. Giegerich, "Fast and effective prediction of microRNA/target duplexes," *RNA*, vol. 10, (no. 10), pp. 1507-17, Oct 2004.

[34] M. Sturm, M. Hackenberg, D. Langenberger, and D. Frishman, "TargetSpy: a supervised machine learning approach for microRNA target prediction," *BMC Bioinformatics*, vol. 11, pp. 292, 2010.

[35] J.M. Brockman, P. Singh, D. Liu, S. Quinlan, J. Salisbury, and J.H. Graber, "PACdb: PolyA Cleavage Site and 3'-UTR Database," *Bioinformatics*, vol. 21, (no. 18), pp. 3691-3, Sep 15 2005.

[36] S. Ambs, R.L. Prueitt, M. Yi, R.S. Hudson, T.M. Howe, F. Petrocca, T.A. Wallace, C.G. Liu, S. Volinia, G.A. Calin, H.G. Yfantis, R.M. Stephens, and C.M. Croce, "Genomic profiling of microRNA

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and messenger RNA reveals deregulated microRNA expression in prostate cancer," *Cancer Res*, vol. 68, (no. 15), pp. 6162-70, Aug 1 2008.

[37] A. Schaefer, M. Jung, H.J. Mollenkopf, I. Wagner, C. Stephan, F. Jentzmik, K. Miller, M. Lein, G. Kristiansen, and K. Jung, "Diagnostic and prognostic implications of microRNA profiling in prostate carcinoma," *Int J Cancer*, vol. 126, (no. 5), pp. 1166-76, Mar 1 2010.

[38] B.S. Taylor, N. Schultz, H. Hieronymus, A. Gopalan, Y. Xiao, B.S. Carver, V.K. Arora, P. Kaushik, E. Cerami, B. Reva, Y. Antipin, N. Mitsiades, T. Landers, I. Dolgalev, J.E. Major, M. Wilson, N.D. Socci, A.E. Lash, A. Heguy, J.A. Eastham, H.I. Scher, V.E. Reuter, P.T. Scardino, C. Sander, C.L. Sawyers, and W.L. Gerald, "Integrative genomic profiling of human prostate cancer," *Cancer Cell*, vol. 18, (no. 1), pp. 11-22, Jul 13 2010.

[39] S. Wach, E. Nolte, J. Szczyrba, R. Stohr, A. Hartmann, T. Orntoft, L. Dyrskjot, E. Eltze, W. Wieland, B. Keck, A.B. Ekici, F. Grasser, and B. Wullich, "MiRNA profiles of prostate carcinoma detected by multi-platform miRNA screening," *Int J Cancer*, Mar 11 2011.

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