Comparative Analysis of Prostate Cancer Gene Regulatory Networks via Hub Type Variation

Pegah Khosravi 1,2, Vahid H. Gazestani 3, Mohammad Akbarzadeh 1, Samira Mirkhalaf 1, Mehdi Sadeghi 2,4, and Bahram Goliaei 1*

1. Department of Bioinformatics, Institute of Biochemistry and Biophysics (IBB), University of Tehran, Tehran, Iran
2. School of Biological Sciences, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran
3. Institute of Parasitology, McGill University, Montreal, Quebec, Canada
4. National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran

Abstract

Background: Prostate cancer is one of the most widespread cancers in men and is fundamentally a genetic disease. Identifying regulators in cancer using novel systems biology approaches will potentially lead to new insight into this disease. It was sought to address this by inferring gene regulatory networks (GRNs). Moreover, dynamical analysis of GRNs can explain how regulators change among different conditions, such as cancer subtypes.

Methods: In our approach, independent gene regulatory networks from each prostate state were reconstructed using one of the current state-of-art reverse engineering approaches. Next, crucial genes involved in this cancer were highlighted by analyzing each network individually and also in comparison with each other.

Results: In this paper, a novel network-based approach was introduced to find critical transcription factors involved in prostate cancer. The results led to detection of 38 essential transcription factors based on hub type variation. Additionally, experimental evidence was found for 29 of them as well as 9 new transcription factors.

Conclusion: The results showed that dynamical analysis of biological networks may provide useful information to gain better understanding of the cell.

Keywords: Gene regulatory networks, Prostate cancer, Transcription factors

Introduction

The complexity and multigenic nature of cancer has necessitated various genome-wide studies to achieve a systems-level understanding of the key genetic mediators involved in prostate cancer 1. Most diseases are due to the collapse of cellular processes together with interaction networks 2. Therefore, exploring the biological network for complex diseases provides an understanding of the functional alterations in chronic diseases 3.

Network-based approaches contain many clinical applications to explore human diseases systematically. A better understanding of the effects of cellular networks on disease progression may lead to the identification of disease genes which, in turn, may offer better targets for drug development 4. One focal point in cancer analysis is the reconstruction of Gene Regulatory Networks (GRN) 5. However, cancer progression is a dynamic process with multiple stages; so, reconstruction of one static GRN may not be informative enough for the inference. Instead, reconstructing stage-specific GRNs during cancer progression and then comparing these GRNs would be beneficial to characterize the main genes and interactions involved in cancer progression.

The availability of genome-wide gene expression data has helped develop various state-of-art GRN reconstruction methods 5-7. These methods seek to identify putative gene regulatory interactions by assuming that alterations in the expression level of a regulator (such as a transcription factor) have a direct effect on the cognate regulated genes.

Empirical evidence of extensive GRN rewiring during cancer progression, along with the availability of GRN reverse engineering approaches, have inspired us to conduct a systematic investigation to characterize the topological changes that occur in a prostate cell’s GRN during cancer progression. Therefore, in this study, an attempt was made to reveal candidate disease-associated genes and biomarkers for prostate cancer progression by integrative gene expression profiling and network analysis at a systematic level. In this way, four stage-specific GRNs were reconstructed.
Materials and Methods

Network reverse engineering approaches

Reverse engineering of GRNs from whole genome data entails deciphering the underlying gene regulatory circuits by observing changes in gene expression profiles. With advances in high-throughput technologies, several computational reverse engineering approaches using different statistical measures have been developed. Through sub-network analysis, it is possible to identify significant genes which were supposed to change their hub type with highly relevant to specific phases of prostate cancer.

Enormous efforts have been made to identify biomarkers for various cancers by the analysis of different transcriptome data. Moreover, there were similar studies for analysis of sub-networks or hub genes which had been helpful for the understanding of the metastasis of cancer at the molecular level. Nonetheless, there are still few studies on identification of prostate cancer biomarkers for disease progression. Therefore, in this study, a new integrative network-based approach was developed to detect party hubs and date hubs based on Degree and BN algorithms during cancer progression.

Our analysis led to identification of 38 important genes putatively involved in prostate cancer. Through extensive literature search, experimental evidences revealed the role of 76.3% of candidate genes in prostate cancer (Table 1). This level of experimental confirmation reflects the high accuracy of the proposed approach.

Our study hereby demonstrates a useful approach for analysis of prostate cancer at the systematic level. For the genome-wide investigations, this will be a fundamental attempt for future development of the translational medical informatics, which lead to better patient diagnostics with high-throughput data through systems biology.

Results

Stage specific network reconstruction of prostate cancer

Using the CLR algorithm, four independent networks related to the four different cell stages were reconstructed (Normal, Adjacent, Tumor, and Metasta-
Comparative Analysis of Gene Regulatory Networks in Prostate Cancer via Hub Type Variation

Table 1. The function of 29 critical transcription factors putatively involved in prostate cancer

| Gene name | Function |
|-----------|----------|
| AR        | Androgen receptor gene transcripts are over-expressed in most metastatic prostate cancers (40). |
| ATF6      | ATF6-mediated apoptosis is reported in many cancers such as prostate cancer (49, 50). |
| SMAD3     | SMAD3 is an essential mediator of tumor suppression and apoptosis in prostate cancer (41). |
| GATA3     | Prostatic GATA3 is involved in androgen regulation of the prostate-specific antigen gene (51). |
| HLF       | HLF is involved in prostate cancer promotion and progression (52). |
| PNBX1     | The Pbx1-HoxC8 heterocomplex causes androgen-independent growth in prostate cancer (53). |
| FOXF1     | FOXF1 has high expression in normal prostate and its expression decreases in prostate cancer (39). |
| TCF21     | TCF21 methylation levels accurately discriminate bladder and prostate cancerous tissues from their normal counterparts (54). |
| STAT1     | Progressive dysregulation of STAT1 in prostate cancer cells contributes to prostate tumor growth (55). |
| EGR3      | EGR3 is significantly over-expressed in prostate cancer and is a candidate prognostic marker of poor outcome prostate cancer (56). |
| PKN6      | In human prostate cancer, up-regulation of PKN6 protein occurs in advanced diseases and correlates with MAPK pathway activation (57). |
| KLF6      | KLF6 controls cell cycle progression and apoptosis and is usually inactivate in many cancers such as prostate, ovary and colon (37, 38). |
| FOXA1     | High-level FOXA1 expression is associated with the development of metastatic prostate cancer and could be used to classify patients who are at higher risk for metastases (58). |
| ELK4      | ELK4 plays important roles in cell growth regulation of prostate cancer cells. The level of the transcript correlates with the progression of the disease (59). |
| HOXC6     | Down-regulation of HOXC6 due to decreased proliferation rates of cell line and the over-expression of it rescues the cells from apoptosis in prostate cancer (60). |
| VDR       | Studies discriminated the impact of VDR ligands upon prostate cancer cell proliferation, differentiation, and apoptosis (42). |
| RARB      | RARB gene methylation in prostate samples is associated with an increased risk of subsequent prostate cancer (61). |
| EZH2      | Over-expression of EZH2 causes invasion and growth of prostate cells. It is also a good biomarker for detection of the problem at an advanced stage (62). |
| TTFAP2A (AP-2) | Cytoplasmic expression of AP-2 is reduced in prostate cancer cells (63). |
| JUNB      | JUNB has an important role in controlling prostate cancer and can be a target for cancer therapy (64). |
| SNA12     | Down-regulation of SNA12 is associated with primary prostate cancers and is a negative regulator of proliferation in the cancer cells (65). |
| ZEB1      | Cancerous phenotype in prostate cancer cells is associated with increased expression of ZEB1 (66). |
| MXD4 (MAD4) | MXD4 which is known to have antitumor properties is significantly up-regulated in treated PC (67). |
| MAZ       | MAZ expression deregulation relates to progression of many cancer types and plays an important role in PCA pathogenesis (68). |
| HOXB13    | Recurrent mutation in HOXB13 associates with an increased risk of hereditary prostate cancer (69). |
| SIM2      | Studies suggested an involvement of SIM2 in prostate tumor cell and cancer progression (70). |
| INSM1     | Investigation showed that INSM1 remarkably up-regulates at the advanced PC stages (71). |
| PLAGL1    | PLAGL1 is a tumor suppressor gene that inhibits growth of tumor cells by controlling apoptosis and cell-cycle progression in prostate cancer (72). |
| FOXC1     | It was indicated that FOXC1 links to androgen-associated growth status of prostate cancer (39). |

Detection of essential transcription factors involved in the prostate cancer

Considering the importance of hub and bottleneck proteins in the structure of GRNs, the 50 highest-ranked genes were identified for each stage-specific GRN based on their degree and bottleneck scores, separately. Top 50 genes were selected based on previous studies that showed the highest percentage of critical proteins found in top 50 ones based on Degree and BN algorithm. Although there were four conditions that resulted in detection of 200 genes, 144 of 200 genes had overlap during various conditions. Consequently, 56 unique candidate genes were selected for further analysis.

In each GRN, these 56 genes were categorized based on their degree and bottleneck scores in four groups: 1) Hub-NonBottleneck: genes with high degrees and low bottleneck scores are putative party hubs; 2) Hub-Bottleneck: genes with high degrees and high bottleneck scores are putative date hubs; 3) NonHub-Bottleneck: genes with low degrees and high bottleneck scores; 4) NonHub-NonBottleneck: genes with low degrees and low bottleneck scores.

The results showed hub type variation for 38 genes across different stages, whereas 18 other genes were functionally conserved as date hubs under all conditions (Table 2). Sub-networks consisting of the first neighborhoods of the 38 critical bottleneck transcription factors were extracted and compared, revealing changes in the...
number of interactions and gene targets between the stages. For some transcription factors such as STAT1, AR, HLF, ZEB1, TCF21, ISL1, KLF6, HOXB13, SIM2 and FOXA1, the number of interactions in the metastasis stage decreased dramatically (Table 3); the interaction numbers of other transcription factors, such as ZNF-529, FOXC1, MNX1, and JUNB, increased considerably in the metastasis stage, indicating network rewiring (Table 3). These 14 transcription factors (Figure 2) showed dramatic changes in their number of interactions (fold change ≥2) during the cancer progression (Table 3).

Discussion

To reconstruct cell stage specific GRNs, an attempt was made to focus on the available comprehensive transcriptome dataset, originally published in 20. This dataset was generated by sampling from four different...
Comparative Analysis of Gene Regulatory Networks in Prostate Cancer via Hub Type Variation

Avicenna Journal of Medical Biotechnology, Vol. 7, No. 1, January–March 2015

Types of prostate tissues including normal cells (Normal), normal cells adjacent to cancer cells (Adjacent), primary tumor cells (Tumor), and metastatic cells (Metastasis). In our approach, genes were analyzed and prioritized based on the transcriptome data. Hence, it was possible to make reliable predictions only for genes with altered expression level across various conditions. To focus on these genes, only up/down-regulated genes were considered (fold change $\geq 2$ and $p<0.05$) in at least one cell stage (978 genes). Also, enrichment of known cancer genes was checked among this set by using a previously curated list of 555 high confidence cancer genes, originally published in 31. 100 genes were collected and identified as mediators in metastatic prostate cancer from 32, and 276 genes were added and annotated as either a cancer pathway or prostate cancer gene in the KEGG database. It was found that the cancer-related genes were about 1.55-fold (hypergeometric two-tailed test, $p=2.52E-6$) and the prostate cancer-related genes were about 2.22-fold enriched (hypergeometric two-tailed test, $p=6.07E-6$) in our selected gene set which were fluctuated during prostate cancer.

To identify master regulators and their associated circuits governing cell-specific behavior in each state, the GRNs of prostate cells were compared in different stages with each other. Because the CLR algorithm merely relies on the similarity of expression patterns to

### Table 2. 56 transcription factors showed different topological characteristics in different stages

| Gene name | N | A | T | M | Gene Name | N | A | T | M |
|-----------|---|---|---|---|-----------|---|---|---|---|
| HOXB13    | PH | DH | PH | DH | ZNF516    | DH | DH | DH | DH |
| ZEB1      | PH | DH | DH | DH | GATA3     | DH | DH | DH | NB |
| SIM2      | PH | DH | DH | NB | ZNF423    | DH | DH | DH | DH |
| TFAP2A    | PH | DH | DH | DH | KLF6      | DH | NN | NN | DH |
| ZNF205    | PH | DH | DH | DH | ZNF91     | DH | DH | DH | DH |
| EZH2      | PH | NN | DH | PH | ETS2      | DH | DH | DH | DH |
| MXD4      | PH | DH | DH | DH | NFI A     | DH | DH | DH | DH |
| ZNF146    | PH | DH | DH | DH | MEIS1     | DH | DH | DH | DH |
| CAMTA1    | PH | NB | NN | DH | SMAD3     | NB | NB | DH | DH |
| MAZ       | PH | DH | DH | DH | MEIS2     | DH | DH | DH | DH |
| PLAGL1    | PH | DH | DH | PH | STAT5A    | DH | DH | DH | DH |
| HOXC6     | DH | PH | DH | PH | PRRX1     | DH | DH | DH | DH |
| FOS       | DH | PH | DH | DH | NHLH2     | DH | NN | DH | DH |
| ELK4      | DH | PH | NN | DH | FOXN3     | DH | DH | DH | DH |
| NKX2-2    | DH | DH | PH | DH | EGR3      | DH | DH | DH | NN |
| MXN1      | NB | NN | PH | DH | ID1       | DH | DH | DH | DH |
| AR        | DH | DH | DH | PH | NRR4A1    | DH | DH | DH | DH |
| PBX1      | DH | DH | DH | PH | NRH1H2    | DH | DH | DH | DH |
| FOXA1     | DH | DH | DH | PH | FOXF1     | DH | DH | DH | NN |
| INSM1     | NB | DH | NN | PH | ATF6      | DH | NB | DH | DH |
| MEF2C     | DH | DH | DH | DH | STAT6     | DH | DH | DH | DH |
| STAT1     | DH | DH | DH | NB | VDR       | DH | DH | NN | DH |
| NR3C1     | DH | DH | DH | DH | FOXC1     | NB | DH | DH | DH |
| HLF       | DH | DH | DH | NB | STAT2     | NB | NB | DH | NB |
| TP63      | DH | DH | DH | NB | JUNB      | NN | DH | DH | DH |
| TCF21     | DH | DH | DH | NB | RARB      | NB | DH | NB | NB |
| ISL1      | DH | NB | DH | NB | SNAI2     | NB | NN | DH | NB |
| EGR1      | DH | DH | DH | DH | ZNF529    | NN | NN | NB | DH |

N) Normal; A) Adjacent; T) Tumor; M) Metastasis; DH) Date Hub; PH) Party Hub; NB) Nonhub-Bottleneck; NN) Nonhub-Nonbottleneck.
infer interactions, constructed networks in this step contain both regulatory interactions (interactions between regulated genes and their putative regulators) as well as interactions between co-regulated genes (non-regulatory interactions). Hereafter, these networks are called co-expression networks. To extract gene regulatory interactions from these networks, only interactions involved at least one human transcription factor were considered and a list of them were extracted from 33,34. These networks are referred to as GRNs.

To predict the key genes in the prostate cancer, an attempt was made to find the stage-specific co-expression networks of prostate cancer for high connectivity (hub) or bottleneck genes 22,23. Hub and bottleneck properties are considered important centrality indices because they are major intersections between clusters in the network and if they are removed from a network, the network will be disrupted 24. Han et al suggested the existence of two types of protein hubs in the protein-protein interaction networks, namely party hubs and date hubs 30. Although both interact with many proteins, the difference is that party hubs are proteins that interact with many other proteins simultaneously, whereas date hubs interact with their partners asynchronously 35. By definition, the bottleneck proteins are responsible for the interconnection of clusters in the network, and thus bottlenecks with high degrees are most likely to be date hubs which contain groups of genes that assist in presenting common functions 33,35. The obtained results recapitulate previous findings in which some active sub-networks contained regulatory interactions were supplanted by new interactions which changed their degrees during different conditions 36.

The result also reflected the high level of rewiring of gene regulatory circuits during cancer progression, as suggested elsewhere 8. As shown in table 3, for example, more than 2-fold decrease in the number of interactions for KLF6 was observed which controlled cell cycle progression and apoptosis. Indeed, experimental data suggest that KLF6 is inactivated in many cancers such as prostate, ovary and colon 37,38. On the other hand, consistent with more than 2-fold increase in the number of interactions (from 14 in normal stage to 33 in the metastasis stage) for FOXC1 (Table 3), it was indicated that this gene is linked to androgen dependent growth of prostate cancer 39.

Our result led to identification of 38 transcription factors which were bottleneck and changed their interaction during cancer progression. Although the functional role of some famous transcription factors such as AR, SMAD3 and VDR are well known as genes linked to prostate cancer 40-42, the 9 transcription factors (CAMTA1, ISL1, MNX1, NHLH2, NKX2-2, STAT2, ZNF146, ZNF205, and ZNF529) are new candidates that may have critical roles in prostate cancer based on topological significance and regulatory changes during cancer progression. Among the remaining 9 transcription factors, 5 of them were associated with other cancer types. ZNF146, CAMTA1, NKX2-2, MNX1, and ISL1 are most prominent in colorectal cancer, neuroblastoma, Ewing’s sarcoma, leukemia and breast cancer, and bladder cancers, respectively 43-48. No evidence could be found to show the relationship between the 4 remaining transcription factors and any type of cancer.

### Conclusion

In this paper, an accurate network-based framework for the analysis of transcriptome data was presented. The analysis of prostate state specific GRNs revealed 38 transcription factors which are critically important for prostate cancer progression. Also, 14 transcription factors which were bottleneck and changed their interaction during cancer progression. Although the functional role of some famous transcription factors such as AR, SMAD3 and VDR are well known as genes linked to prostate cancer 40-42, the 9 transcription factors (CAMTA1, ISL1, MNX1, NHLH2, NKX2-2, STAT2, ZNF146, ZNF205, and ZNF529) are new candidates that may have critical roles in prostate cancer based on topological significance and regulatory changes during cancer progression. Among the remaining 9 transcription factors, 5 of them were associated with other cancer types. ZNF146, CAMTA1, NKX2-2, MNX1, and ISL1 are most prominent in colorectal cancer, neuroblastoma, Ewing’s sarcoma, leukemia and breast cancer, and bladder cancers, respectively 43-48. No evidence could be found to show the relationship between the 4 remaining transcription factors and any type of cancer.
factors were identified to be linked putatively to prostate cancer metastasis stage, so they would be used as key factors for future research in the field of cancer studies. Additionally, experimental evidences revealed the role of 29 of candidate transcription factors in prostate cancer.

The low number of predictions and high degree of overlap with previously known events in the prostate cancer demonstrate the high efficiency of our approach. In addition, the low number of predicted gene sets makes it easy to design follow up experiments to validate the results. In this study, it is believed that the results may provide critical information to gain better understanding of networks dynamics in the cell through complex diseases such as cancer.

Acknowledgement

Pegah Khoosravi has been supported by the School of Biological Sciences of Institute for Research in Fundamental Sciences (IPM), Vahid H. Gazestani has been supported by CIHR Systems Biology Fellowship. The authors would like to thank Dr. Gary Bader and Dr. Juri Reimand in the Bader’s lab for their invaluable insights.

References

1. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med 2004;10(8):789-799.
2. Goh KI, Cusick ME, Valle D, Childs B, Vidal M, Barabasi AL. The human disease network. Proc Natl Acad Sci USA 2007;104(21):8685-8690.
3. Paik H, Heo HS, Ban HJ, Cho SB. Unraveling human protein interaction networks underlying co-occurrences of diseases and pathological conditions. J Transl Med 2014;12:99.
4. Barabasi AL, Gulbahce N, Loscalzo J. Network medicine: a network-based approach to human disease. Nat Rev Genet 2011;12(1):56-68.
5. Margolin AA, Nemenman I, Basso K, Wiggins C, Stolovitzky G, Dalla Favera R, et al. ARACNE: an algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context. BMC Bioinformatics 2006;7 Suppl 1:S7.
6. Faith JJ, Hayete B, Thaden JT, Mogno I, Wierzbowski J, Cottarel G, et al. Large-scale mapping and validation of Escherichia coli transcriptional regulation from a compendium of expression profiles. PLoS Biol 2007;5(1):e26.
7. Butte AJ, Kohane IS. Mutual information relevance networks: functional genomic clustering using pairwise entropy measurements. Pac Symp Biocomput 2000;418-429.
8. Huang S, Ingber DE. A non-genetic basis for cancer progression and metastasis: self-organizing attractors in cell regulatory networks. Breast Dis 2006;26:27-54.
9. Kallioniemi O. Functional genomics and transcriptomics of prostate cancer: promises and limitations. BJU Int 2005;96 Suppl 2:10-15.
10. Jiang J, Cui W, Vongsangnak W, Hu G, Shen B. Post-gene-profiling studies functional characterization of prostate cancer risk loci. BMC Genomics 2013;14 Suppl 8:89.
11. Tang Y, Yan W, Chen J, Luo C, Kaipia A, Shen B. Identification of novel microRNA regulatory pathways associated with heterogeneous prostate cancer. BMC Syst Biol 2013;7 Suppl 3:S6.
12. Ideker T, Sharan R. Protein networks in disease. Genome Res 2008;18(4):644-652.
13. Taylor IW, Linding R, Warde-Farley D, Liu Y, Pesquita C, Faria D, et al. Dynamic modularity in protein interaction networks predicts breast cancer outcome. Nat Biotechnol 2009;27(2):199-204.
14. Brahmachari SK. Introducing the medical bioinformatics in Journal of Translational Medicine. J Transl Med 2012;10:202.
15. Friedman N. Inferring cellular networks using probabilistic graphical models. Science 2004;303(5659):799-805.
16. Schaffer J, Strimmer K. An empirical Bayes approach to inferring large-scale gene association networks. Bioinformatics 2005;21(6):754-764.
17. Butte AJ, Tamayo P, Slonim D, Golub TR, Kohane IS. Discovering functional relationships between RNA expression and chemotherapeutic susceptibility using relevance networks. Proc Natl Acad Sci USA 2000;97(22):12182-12186.
18. Basso K, Margolin AA, Stolovitzky G, Klein U, Dalla-Favera R, Califano A. Reverse engineering of regulatory networks in human B cells. Nat Genet 2005;37(4):382-390.
19. Marbach D, Costello JC, Kuffner R, Vega NM, Prill RJ, Camacho DM, et al. Wisdom of crowds for robust gene network inference. Nat Methods 2012;9(8):796-804.
20. Chandran UR, Ma C, Dhir R, Bisceciglia M, Lyons-Weiler M, Liang W, et al. Gene expression profiles of prostate cancer reveal involvement of multiple molecular pathways in the metastatic process. BMC Cancer 2007;7:64.
21. Smyth GK. Limma: linear models for microarray data. In: Huber W, editor. Bioinformatics and computational biology solutions using R and bioconductor. New York: Springer; 2005. p. 397-420.
22. Freeman LC. Set of measures of centrality based on betweenness. Sociometry 1977;40(1):35-41.
23. Girvan M, Newman MEJ. Community structure in social and biological networks. Proc Natl Acad Sci USA 2002;99(12):7821-7826.
24. Yu HY, Kim PM, Sprecher E, Trifonov V, Gerstein M. The importance of bottlenecks in protein networks: Core modules are over-represented in protein complexes. Genome Res 2005;15(12):1904-1915.
25. Ideker T, KDMG. Protein networks in disease. Genome Res 2005;15(6):1425-1432.
26. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13(11):2498-2504.
27. Cline MS, Smoot M, Cerami E, Kuchinsky A, Landys N, Workman C, et al. Integration of biological networks and gene expression data using Cytoscape. Nat Protoc 2007; 2(10):2366-2382.
28. Watts DJ, Strogatz SH. Collective dynamics of ‘small-world’ networks. Nature 1998;393(6684):440-442.
29. Barabasi AL, Albert R. Emergence of scaling in random networks. Science 1999;286(5439):509-512.
30. Han JD, Bertin N, Hao T, Goldberg DS, Berriz GF, Zhang LV, et al. Evidence for dynamically organized modularity in the yeast protein-protein interaction network. Nature 2004;430(6995):88-93.
31. Reimand J, Bader GD. Systematic analysis of somatic mutations in phosphorylation signaling predicts novel cancer drivers. Mol Syst Biol 2013;9:637.
32. Ergun A, Lawrence CA, Kohanski MA, Brennan TA, Collins JJ. A network biology approach to prostate cancer. Mol Syst Biol 2007;3:82.
33. Vaquerizas JM, Kummerfeld SK, Teichmann SA, Luscombe NM. A census of human transcription factors: function, expression and evolution. Nat Rev Genet 2009;10(4):252-263.
34. Kummerfeld SK, Teichmann SA. DBD: a transcription factor prediction database. Nucleic Acids Res 2006;34:D74-D81.
35. Dunn R, Dudbridge F, Sanderson CM. The use of edge-betweenness clustering to investigate biological function in protein interaction networks. BMC Bioinformatics 2005;6:39.
36. Luscombe NM, Babu MM, Yu H, Snyder M, Teichmann SA, Gerstein M. Genome analysis of regulatory network dynamics reveals large topological changes. Nature 2004;431(7006):308-312.
37. Chen CH, Huang PH, Chu PC, Chen MC, Chou CC, Wang D, et al. Energy restriction-mimetic agents induce apoptosis in prostate cancer cells in part through epigenetic activation of KLFL6 tumor suppressor gene expression. J Biol Chem 2011;286(12):9968-9976.
38. Narla G, Heath KE, Reeves HL, Li D, Giono LE, Kimmelman AC, et al. KLFL6, a candidate tumor suppressor gene mutated in prostate cancer. Science 2001;294(5551):2563-2566.
39. van der Heul-Nieuwenhuijsen L, Dits NF, Jenster G. Gene expression of forkhead transcription factors in the normal and diseased human prostate. BJU Int 2009;103(11):1574-1580.
40. Mazaris E, Tsiotras A. Molecular pathways in prostate cancer. Nephrourol Mon 2013;5(3):792-800.
41. Reed JA, Lin Q, Chen D, Mian IS, Medrano EE. SKI pathways inducing progression of human melanoma. Cancer Metastasis Rev 2005;24(2):265-272.
42. Skinner HG, Schwartz GG. Serum calcium and incident and fatal prostate cancer in the National Health and Nutrition Examination Survey. Cancer Epidemiol Biomarkers Prev 2008;17(9):2302-2305.
43. Ferbus D, Bovin C, Validire P, Goubin G. The zinc finger protein OZF (ZNF146) is overexpressed in colorectal cancer. J Pathol 2003;200(2):177-182.
44. Henrich KO, Fischer M, Mertens D, Benner A, Wiedemeyer R, Brors B, et al. Reduced expression of CAMTA1 correlates with adverse outcome in neuroblastoma patients. Clin Cancer Res 2006;12(1):131-138.
45. Smith R, Owen LA, Trem DJ, Wong JS, Whangbo JS, Golub TR, et al. Expression profiling of EWS/FLI identifies NKX2.2 as a critical target gene in Ewing’s sarcoma. Cancer Cell 2006;9(5):405-416.
46. Nagel S, Kaufmann M, Scherr M, Drexler HG, MacLeod RA. Activation of HLXB9 by juxtaposition with MYB via formation of t(6;7)(q23;q36) in an AML-M4 cell line (GDM-1). Genes, Chromosomes Cancer 2005;42(2):170-178.
47. Lian ZQ, Wang Q, Li WP, Zhang AQ, Wu L. Screening of significantly hypermethylated genes in breast cancer using microarray-based methylated-CpG island recovery assay and identification of their expression levels. Int J Oncol 2012;41(2):629-638.
48. Kim YJ, Yoon HY, Kim JS, Kang HW, Min BD, Kim SK, et al. HOXA9, ISL1 and ALDH1A3 methylation patterns as prognostic markers for nonmuscle invasive bladder cancer: array-based DNA methylation and expression profiling. Int J Cancer 2013;133(5):1135-1142.