Original Article

Analysis of Candidate Genes Has Proposed the Role of Y Chromosome in Human Prostate Cancer

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Abstract

Background: Prostate cancer, a serious genetic disease, has known as the first widespread cancer in men, but the molecular changes required for the cancer progression has not fully understood. Availability of high-throughput gene expression data has led to the development of various computational methods, for identification of the critical genes, have involved in the cancer.

Methods: In this paper, we have shown the construction of co-expression networks, which have been using Y-chromosome genes, provided an alternative strategy for detecting of new candidate, might involve in prostate cancer. In our approach, we have constructed independent co-expression networks from normal and cancerous stages have been using a reverse engineering approach. Then we have highlighted crucial Y chromosome genes involved in the prostate cancer, by analyzing networks, based on party and date hubs.

Results: Our results have led to the detection of 19 critical genes, related to prostate cancer, which 12 of them have previously shown to be involved in this cancer. Also, essential Y chromosome genes have searched based on reconstruction of sub-networks which have led to the identification of 4 experimentally established as well as 4 new Y chromosome genes might be linked putatively to prostate cancer.

Conclusion: Correct inference of master genes, which mediate molecular, has changed during cancer progression would be one of the major challenges in cancer genomics. In this paper, we have shown the role of Y chromosome genes in finding of the prostate cancer susceptibility genes. Application of our approach to the prostate cancer has led to the establishment of the previous knowledge about this cancer as well as prediction of other new genes.

Keywords: Co-expression networks; expression data; prostate cancer; reverse engineering approach

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Introduction

Prostate cancer has known a complex polygenic disorder which would be one of the most known cause of mortality in men [1]. Although recent studies have identified a number of variants, gene fusions, and expression signatures have affiliated with prostate cancer, then identification and characterization of genes that have involved in this cancer, has remained as a formidable challenge [2].

The complexity and multigenic nature of cancer has caused various genome-wide studies, have been achieving a systems-level understanding of the key genetic mediators, involved in prostate cancer [3]. One focal point in cancer analysis would be the reconstruction of co-expression networks. When accurate, co-expression networks have represented the key mediators that have involved in a specific process. The availability of the genome-wide gene expression data has helped the development of various state-of-art co-expression networks reconstruction methods [4-6]. Taking a systems-wide approach, we have reconstructed two stage-specific co-expression networks, based on a comprehensive prostate cancer gene expression

dataset containing 171 different samples monitoring gene expression in two different cell states.

The Y chromosome would be the male-specific chromosome in the human genome. It has played critical vital functions in male-specific organs, such as testis and prostate glands [7]. There were evidences indicating that many forms of tumors have associated with structural and gene expression variations of the Y chromosome [8]. Previous studies have shown that there were about 60 genes existing, but in the present day human Y chromosome have identified as the survivors of at least a set of 1500 genes that have assumed to exist in the early proto-Y element [7, 9]. Although the involvement of Y chromosome has reported for association with prostate cancer [10, 11], currently there was little information regarding the contribution of the Y linked genes, with progression of prostate cancer.

In this paper we have tried to address this problem to identify candidate genes on the Y chromosome that have involved in prostate cancer.

Our analysis has led to identification of both well-established and novel genes, have involved in the prostate cancer. Additionally, we have identified 27 important genes putatively involved in prostate cancer. After extensive literature search, we have found that for 16 of our candidate genes (about 60%), there was experimental evidences suggesting a role in prostate cancer.

Materials and Methods

Network reverse engineering approaches

Reverse engineering of co-expression networks, from the whole genome data, has entailed deciphering the underlying gene regulatory circuits, observing the changes in gene expression profiles. After advances in high-throughput technologies, several computational reverse engineering has approached the different statistical measures [12-15], including information-theoretic network inference methods, which has identified connections, between genes, to approach the quantity of common information to any pair of genes. Based on the Dialogue on Reverse Engineering Assessment and Methods 5 (DREAM5) challenge, the context likelihood relevance (CLR) algorithm by Faith *et al.* [4] had the best performance among information theory based approaches [16].

Briefly, CLR has evaluated an interaction between two genes as significant by estimation the significance of their mutual information (MI) value, against a MI values background distribution of every other pair, which have involved one of the two genes of interest. In this way, the significance level has dynamically determined for each interacting pair, according to their expression profiles. To give a gene expression dataset and the significance scores, have calculated by the CLR algorithm, the corresponding empirical false discovery rate (FDR) could be estimated by running the algorithm on randomly shuffled datasets.

In this study, we have reconstructed two co-expression networks using CLR with an FDR threshold of 0.05.

Y chromosome genes

Through a series of expression studies that have conducted on all 60 genes (locus) on the human Y chromosome [7], at the initial phase of this study, we have retrieved all genes that interact with Y chromosome genes from Information Hyperlinked over Proteins (iHOP) database [17]. Overall, we have identified 471 genes in the entire human genome that interact with genes on the Y chromosome.

Prostate cancer microarray data

Prostate cancer microarray data have downloaded from the Gene Expression Omnibus (GEO) database, accession number GDS2545 [18]. This dataset has contained 171 samples, including samples from normal prostate tissue free of any pathology (Normal with 18 samples), normal prostate tissue adjacent to tumors (Adjacent with 63 samples), primary prostate tumor tissue (Tumor with 65 samples), and metastatic prostate cancer (Metastasis with 25 samples). We have considered Normal and Adjacent tissues as normal prostate tissues and Tumor and Metastasis as cancerous prostate tissues. Microarray data have preprocessed and analyzed, using the LIMMA package in R [19] which has originally developed for differential expression analysis of microarray data. Quantile normalization and a moderated t-statistic have used to find differentially expressed genes. More detailed descriptions of the methods could be found in the original publications.

Network topological analysis

Our analysis had two distinct objectives. The first was for identifying the most likely candidate oncogene among the genes in the reconstructed networks via topological analysis. The second one was for evaluating the Y-chromosome genes that

Table 1. 19 of 22 genes have shown different topological characteristics in different networks. Investigations have shown that 12 genes have putatively involved in prostate cancer.

Gene name	Normal	Cancerous	Gene functions
GAPDH	Party hub	Date hub	Expression level of GAPDH is significantly different between cancer and normal tissue, so this gene is a suitable denominator for gene expression studies in prostate cancer [34].
FGFR1	Party hub	Date hub	Expression of FGFR1 is closely linked to prostate cancer progression [35].
RB1	Party hub	NA	The loss of the RB1 gene is an important event in prostate cancer tumorigenesis [36].
MYB	Party hub	Date hub	MYB is amplified in prostate cancer [37].
USP9X	Party hub	Date hub	Over-expression of USP9X was reported in breast cancer tissue compared to adjacent normal tissue [38].
SF1	Party hub	NA	Studies showed the importance of SF1 dosage during tumorigenesis of adrenal cortex [39].
KLK3	Date hub	Party hub	KLK3 and its encoded protein (PSA) are related to prostate cancer and used as a biomarker for this disease [40].
SMAD3	Date hub	Party hub	SMAD3 is an important co-regulator for the androgen-signaling pathway and has a positive role in prostate cancer growth [41].
BCR	Date hub	Party hub	BCR–ABL1 gene fusion is the underlying aberration that cause to 10% of all leukemia [42].
HEXA	NA	Party hub	Activity increasement of HEXA were reported in various types of human cancer such as ovarian [43].
CD44	Date hub	Party hub	CD44 is a metastasis suppressor gene for prostate cancer and its expression level is down-regulated during prostate cancer progression [44].
SFN	NA	Party hub	Some of tumor suppressor gene such as SFN was highly methylated in prostate cancer [45].
HMGB2	NA	Party hub	AR signaling is modulated by AR cofactors such as HMGB2, so the modification of this cofactor may cause androgen-dependent PCa to gain castration-resistant status [46].
IL10RB	NA	Party hub	There is a strong association between the IL10RB SNPs and benign prostate hyperplasia in Korean population [47].
FAS	Date hub	NA	FAS-mediated programmed cell death correlate with the clinical stage of tumors in prostate cancer [48].
TNFRSF25	NA	Date hub	Studies related to bladder cancer showed the higher rates of methylation for TNFRSF25 in malignant than in normal Urothelial tissue [49].
GPI	NA	Date hub	GPI influence tumor growth and promoting cell motility and proliferation [50].
UBE3A	NA	Date hub	UBE3A involves in prostate and mammary gland development. Down-regulation of this gene was reported in prostate cancer compared with normal tissue [51].
OLFM1	NA	Date hub	OLFM1 protein was significantly up-regulated in lung carcinoma than in normal lung tissues [52].
ACTB	Date hub	Date hub	-
MAOA	Date hub	Date hub	-
PTPRC	Date hub	Date hub	-

NA: Neither Party hub nor Date hub

involved in prostate cancer, based on whether they interact with the identified candidate(s) in the reconstructed sub-networks.

To predict the key genes in the prostate cancer, we have searched in the stage-specific co-expression networks of prostate cancer for genes which either had a high number of connections or were bottleneck [20, 21]. The bottleneck genes were important, because if they have removed from a network, the network would be disrupted, as they are

major intersections between clusters in the network [22]. To find such genes, we have topologically analyzed both constructed networks, following the same rules, have been using cyto-Hubba package. [23].

Results

Stage specific network reconstruction of prostate cancer

For the first step, we have reconstructed two independent co-expression networks for the normal and cancerous tissues using the CLR algorithm. We have considered 310 genes of the 471 genes extracted from iHOP due to the availability of expression data from our transcriptomics dataset. The resulting normal network that has contained 2147 interactions, and the cancerous network contained 2201 interactions have used for further analysis. Additionally, topological analysis of the networks has revealed that both networks exhibit the small-word property [24] and scales-free architecture [25] (Figure 1). Both reconstructed networks have mainly composed of the same set genes; however the conserved interactions among these two networks were very low.

Detection of essential genes involved in the prostate cancer

To consider the importance of hub and bottleneck proteins in the structure of co-expression networks, the 10 highest-ranked genes have identified for each stage-specific network based on their degree and bottleneck scores, separately. In total, 22 candidate genes have selected for further analysis.

In each network, we have categorized these 22 genes based on their degree and bottleneck scores in two groups: 1) Hub-NonBottleneck: genes with high degrees and low bottleneck scores, were putative party hubs [26]; 2) Hub-Bottleneck: genes with high degrees and high bottleneck scores, were putative date hubs [26].

The results have shown hub type variation for 19 genes across different tissues, whereas 3 other genes have functionally conserved as date hubs under both conditions (Table 1). We have considered these 19 genes as defined critical genes for further analysis.

Detection of Y chromosome genes involved in the prostate cancer

For the second step, due to detection of the Y chromosome genes that interact with 19 defined critical genes, we have extracted a sub-network

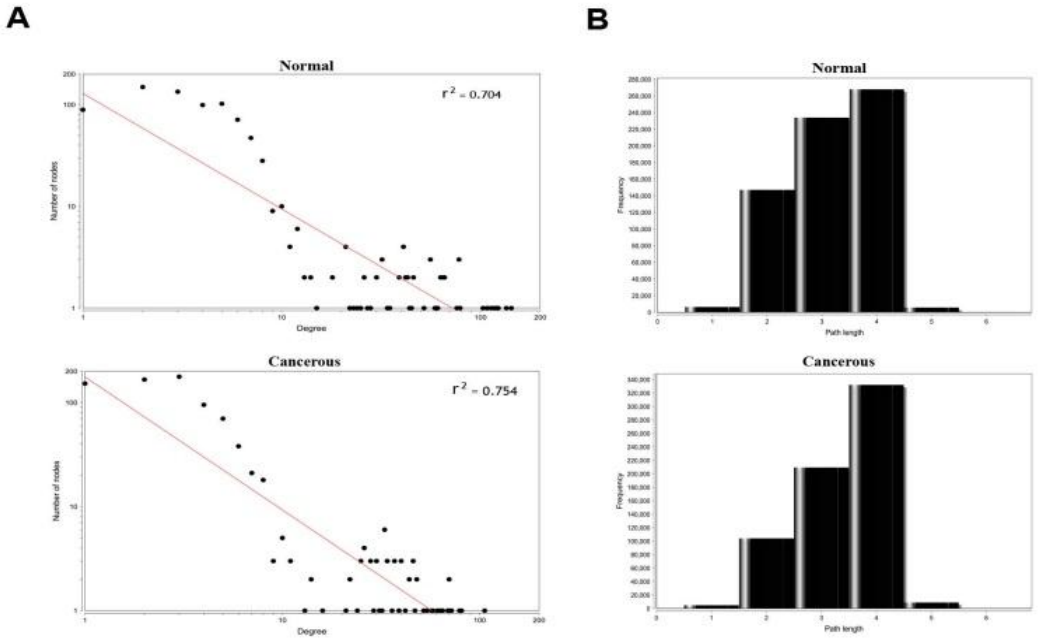

Figure 1. Co-expression Network architecture. Both networks (normal and cancerous network) have followed the well-known characteristics of most biological networks, scales-free architecture (A) defined as few highly connected genes (hubs) that link the other less connected genes to the network and small-word property (B) which meant any two genes in the network could be connected by relatively short paths through all interactions.

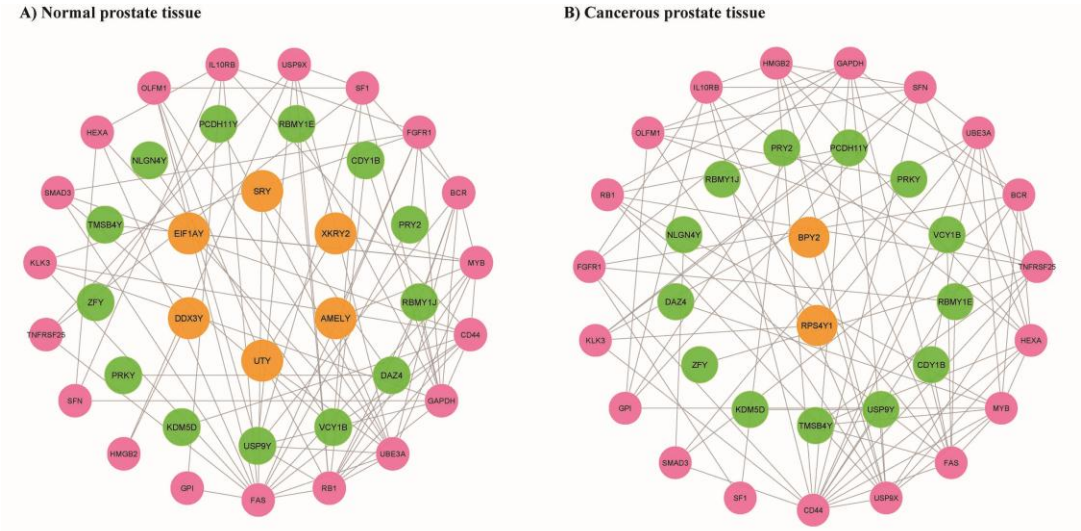

Figure 2. Comparison of normal and cancerous prostate network in terms of interactions among 19 defined critical genes, and Y chromosome genes. Both sub-networks have extracted from constructed co-expression networks based on 19 defined genes and Y chromosome genes. The networks have contained genes from normal prostate tissue (A) and cancerous prostate tissue (B). The 19 defined critical genes have colored in pink and the Y chromosome genes colored in green. Orange nodes in the sub-networks were the genes that have rewired during cancer progression.

using these critical genes and 60 genes on the Y chromosome. The sub-networks have extracted and visualized by Cytoscape software (Figure 2). According to Figure 2, all of the 19 defined critical genes had interactions with 19 genes of 60 genes on the Y chromosome in the normal sub-network while the number of Y chromosome genes in cancerous network decreased to 15 genes of 60 genes. Closer investigation have shown some genes such as SRY, XKRY2, AMELY, UTY, DDX3Y and EIF1AY of the normal network have substituted by BPY2 and RPS4Y1 in the cancerous network (Figure 2). Topological comparison of these two sub-networks have recapitulated the previous findings about extensive networks rewiring [27]. We have done some literature search about these 8 genes. Among the 8 genes, we have identified 4 genes associated with prostate cancer. Although BPY2, UTY, SRY and EIF1AY would be the most prominent in prostate cancer [11, 28], we could not find any evidence to show the relationship between the 4 remaining genes such as RPS4Y1, AMELY, XKRY2 and DDX3Y to any type of cancer.

Discussion

To reconstruct cell stage specific co-expression networks, we have focused on the available comprehensive transcriptome dataset, originally published in [18]. In our approach, genes have analyzed and prioritized based on the transcriptome data. Hence, we were able to make reliable predictions only for genes with altered expression level across normal and cancerous conditions. To focus on these genes, we have considered only genes that have up-/ down-regulated (fold change≥1.5 and p-value<0.05) in cancerous stage and had interaction with Y chromosome genes (310 genes). Although most of the computational have approached to identify group of genes that have significantly up-/down regulated during cancer progression, we have believed in complementary analysis to identify critical genes which involved in cancer. We have checked for enrichment of known cancer genes among this set by using a previously curated list of 555 high confidence cancer genes, originally published in [29]. We have also collected 100 genes identified as mediators in metastatic prostate cancer from [30], and we have added 276 genes annotated as either a cancer pathway or prostate cancer gene in the KEGG database.

To identify master genes and their associated interactions governing cell-specific behavior in normal and cancerous state, we have compared the networks of prostate cells in both stages with each other.

Han *et al.* suggested the existence of two types of protein hubs in the protein-protein interaction networks: party hubs and date hubs [26]. Although both interactions with many proteins, the difference was that party hubs would be proteins that interact with many other proteins simultaneously, whereas date hubs interacted with their partners asynchronously [26]. By definition, the bottleneck proteins were responsible for the inter connection of clusters in the network, and thus bottlenecks with high degrees were most likely date hubs which contain groups of genes that assist to present common functions [22, 31]. The obtained results have recapitulated previous findings which have demonstrated some active sub-networks contained regulatory interactions supplant by new interactions and changed their degrees during different conditions [32].

Our analysis has led to the identification of both well-established and novel genes involved in this type of cancer. Our result has led to the identification of 27 important genes putatively involved in prostate cancer. Additionally, we have identified 19 of 27 genes that are bottleneck and changed their interaction during cancer progression. Although the functional role of 12 of 19 genes were well known as critical genes for prostate cancer, the remaining 7 genes (USP9X, SF1, BCR, HEXA, TNFRSF25, GPI and OLFM1) were new candidates that might have critical roles in prostate cancer based on topological significance and regulatory changes during cancer progression (Table 1). We have identified these genes associated with other cancer types such as breast, leukemia, bladder and lung (Table 1 for more details). We have also shown that in addition to the 4 well known Y chromosome gene (BPY2, SRY, UTY and EIF1AY), 4 other Y chromosome genes such as RPS4Y1, AMELY, XKRY2 and DDX3Y have rewired and thus predicted these to have important roles in prostate cancer.

Conclusion

In this paper, we have presented an accurate network-based approach for the analysis of transcriptome data. The analysis of prostate state specific co-expression networks has revealed that for 16 of our candidate genes, there were experimental evidences regarding to their role in prostate cancer.

Additionally, we have found that about 85% of our candidate genes to be linked to various cancers, so they would be used as key factors for future research in the field of cancer studies.

The low numbers of predictions and high degree of overlap with previously known events have demonstrated the high efficiency of our approach. In addition, the low number of predicted gene sets, has made it easy for designing follow up experiments to validate results.

For the genome-wide investigations, this would be a fundamental challenge for future development of the translational medical informatics, yielding new potential drug target candidates [33].

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Conflict of Interest

The authors have declared that they had no competing interests.

Authors' Contribution

PK and VHG have conceived and designed of the study. PK, JZ, SM and MA have analyzed and interpreted the data. PK has prepared the initial manuscript. BG and MS have headed the research program. All authors have made substantial written contributions to the manuscript, and have given approval to the final version presented here.

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