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Research Article

Bioinformatics analysis of the gene expression profile in Bladder carcinoma

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Abstract

Bladder carcinoma, which has the ninth highest incidence among malignant tumors in the world, is a complex, multifactorial disease. The malignant transformation of bladder cells results from DNA mutations and alterations in gene expression levels. In this work, we used a bioinformatics approach to investigate the molecular mechanisms of bladder carcinoma. Biochips downloaded from the Gene Expression Omnibus (GEO) were used to analyze the gene expression profile in urinary bladder cells from individuals with carcinoma. The gene expression profile of normal genomes was used as a control. The analysis of gene expression revealed important alterations in genes involved in biological processes and metabolic pathways. We also identified some small molecules capable of reversing the altered gene expression in bladder carcinoma; these molecules could provide a basis for future therapies for the treatment of this disease.

Keywords: Bladder carcinoma, differential expression, expression profile, small molecular mimic, susceptibility forecast.

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Introduction

Bladder carcinoma is one of the most common malignant tumors and has a high death rate in China (Dai *et al.*, 2011). World-wide, bladder carcinoma has the ninth highest incidence among malignant tumors (Parkin, 2008). Bladder carcinoma results from cumulative, long-term interactions between genetic and environmental elements and is a complex, multifactorial process involving gene mutations and progressive cellular damage. Bladder carcinoma is much more common in men than in women and predisposing factors include cigarette smoking, long-term contact with chemical products and race (Parkin, 2008).

The malignant transformation of bladder cells originates in DNA mutations and alterations in gene expression. Most studies of the etiology of bladder carcinoma have focused on genetic mutations, particularly in oncogenes such as H-Ras, C-Myc, HER-2, BcL-2, erbB-2 and fibroblast growth factor receptor 3 (FGFR3) (Sanchez-Carbayo and Cordon-Cardo, 2007; Cordon-Cardo, 2008), as well as the inactive of anti-oncogenes and the deletion or loss of heterozygosity (LOH) in associated chromosomal regions for genes such as p53, pRb, p21 and p16 (Shariat *et al.*, 2004). The invasiveness and metastasis of bladder carcinoma involve the abnormal expression of growth factors and related receptors such as vascular endothelial growth factor (VEGF) (Wang *et al.*, 2000) and fibroblast growth factor receptor (FGFR) (Knowles, 2008).

A detailed understanding of multi-gene hereditary diseases, such as cancer, is no longer feasible with traditional single gene studies but requires more powerful tools such as large-scale gene expression analysis provided by recent technological advances. The application of such technologies to the study of bladder carcinoma should be useful in identifying new tumor marker genes that will allow the early diagnosis of bladder cancer.

In this study, we used the gene expression profile of GSE27448 to investigate differential gene expression in bladder carcinoma and to search for small molecules that may reverse the altered gene expression. The availability of high-throughput gene expression data and their analysis by computational bioinformatics may be useful in identifying molecules that could provide a basis for developing new therapies for treating bladder carcinoma.

Materials and Methods

Gene expression in bladder carcinoma cells and normal cells

To investigate the differential gene expression in bladder carcinoma compared to normal cells and the possible mechanisms involved in the initiation of bladder cancer, we analyzed the gene expression profile of bladder carcinoma and normal cells available from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/); accession number:

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GSE27448). This dataset was deposited by Zaravinos *et al.* (2011) and obtained by cell culture, RNA extraction and individual on-chip analysis. The dataset contains five normal cell chips and nine bladder carcinoma cell chips. The platform used was GPL2895 (GE Healthcare/Amersham Biosciences CodeLink Human Whole Genome Bio array). The original files and the platform probe annotation files were downloaded.

Identification of differentially expressed genes (DEGs)

The original data were classified as bladder carcinoma and control (*i.e.*, normal) groups and were analyzed using R software (v.2.13.0) (R Development Core Team, 2008). Initially, the RMA (robust multichip averaging) method (Irizarry *et al.*, 2003) was applied to normalize the data on different chips, and then Limma (Smyth, 2004), a linear regression model software, was used to compare the differential expression on different classes of chips. A value of $p < 0.05$ was used as the cut-off criterion to select genes that were differentially expressed in bladder carcinoma relative to the control group.

Gene ontology (GO) analysis of DEGs

To investigate the DEGs at a functional level, DAVID (Database for Annotation, Visualization and Integrated Discovery) was used to cluster the genes according to the Gene Ontology (GO) (Ashburner *et al.*, 2000) categories of cellular component, biological process and molecular function (Huang da *et al.*, 2009a,b).

Pathway enrichment analysis

To gain more insights of the changes at a functional level, we investigated the dysregulated biological pathways in bladder carcinoma. All of the metabolic and non-metabolic pathways available from the canonical KEGG PATHWAY DATABASE were used as DAVID inputs for KEGG PATHWAY cluster analysis (Huang da *et al.*, 2009a,b). A value of $p < 0.1$ and at least two DEGs contained in a pathway were chosen as the cut-off criteria.

Identification of small molecules

The connectivity map (CMap) database, which contains whole genomic expression profiles for small active molecular inferences, consists 6,100 classes of small molecular interference experiments and 7,056 expression profiles (Lamb *et al.*, 2006). We analyzed genes that were differentially expressed between normal and bladder carcinoma cells and contrasted them with genes involved in small molecular interference in the CMap database in an attempt to identify small molecules associated with these DEGs. The DEGs were classified into up-regulated and down-regulated groups and the top 500 significant probes in each group were chosen for GSEA (Gene Set Enrichment Analysis)analysis; these were then compared to the DEGs after small molecule treatment. Finally, the enrichment values were calculated. These values varied between -1 and 1 and reflected the similarity among the genes: values closer to -1 indicated greater similarity between the genes, *i.e.*, the small molecule could imitate the status of normal cells, whereas values closer to 1 indicated that the small molecule could imitate the status of bladder carcinoma cells.

Results

Identification of DEGs

The classic *t*-test in the Limma package was used to analyze the gene expression profiles of bladder carcinoma and normal cells and identify the DEGs in bladder carcinoma cells. A value of $p < 0.05$ was used as the significant threshold for DEGs. Based on these criteria, 6562 gene probes (corresponding to 2779 genes) were found to have an altered expression.

GO clustering of DEGs

Functional classification of these 2779 DEGs with the online biological classification tool DAVID and a statistical cut-off criterion of $p < 0.001$ indicated significant enrichment of these genes in various GO categories. Table 1 shows that in bladder carcinoma DEGs were enriched in cellular components related to the cytoskeleton and loco-

Table 1 - Cellular component clusters of DEGs.

Term	p value
GO:0030018~Z disc	5.05E-05
GO:0031674~I band	1.89E-04
GO:0043228~non-membrane-bounded organelle	4.55E-04
GO:0043232~intracellular non-membrane-bounded organelle	4.55E-04
GO:0030529~ribonucleoprotein complex	9.57E-04
GO:0030016~myofibril	0.001311
GO:0005840~ribosome	0.001408
GO:0005730~nucleolus	0.002054
GO:0034702~ion channel complex	0.003021
GO:0043292~contractile fiber	0.00483
GO:0000792~heterochromatin	0.00607
GO:0042383~sarcolemma	0.006176
GO:0005887~integral to plasma membrane	0.006652
GO:0044449~contractile fiber part	0.007309
GO:0044459~plasma membrane part	0.007358
GO:0031981~nuclear lumen	0.007461
GO:0031226~intrinsic to plasma membrane	0.008345
GO:0034703~cation channel complex	0.008574
GO:0022625~cytosolic large ribosomal subunit	0.009238
GO:0030017~sarcomere	0.009584

motion (Z discs, I band, myofibrils, contractile fibers, sarcolemma and sarcomere) and signal transduction. There were also changes in cellular components related to protein expression, *e.g.*, the ribonucleoprotein complex, ribosomes, nucleoli, heterochromatin, nuclear lumen and cytosolic large ribosomal subunit.

Table 2 shows the clusters obtained when these DEGs were classified according to biological process ($p < 0.01$). This analysis revealed inter- and intracellular changes in components related to transportation, such as the regulation of intracellular transport, protein localization, the regulation of nucleocytoplasmic transport, the regulation of intracellular protein transport, negative regulation of intracellular transport and negative regulation of nucleocytoplasmic transport. These changes meant that signal molecules could not be transported to their target site, thus altering intercellular signal transduction. Similar changes were observed in other biological processes, such as intracellular signaling cascades, small GTPase-mediated signal transduction and the protein kinase cascade. Other changes affected protein translation and post-translational modifications, such as amino acid auto-phosphorylation, translation, post-translational elongation, protein complex assembly and protein complex biogenesis. Biological processes related to cell development, *e.g.*, peripheral nervous system development, auditory receptor cell differentiation, mechanoreceptor differentiation and auditory receptor cell development, were also affected.

Table 3 shows the DEGs clustered according to molecular function ($p < 0.01$). The changes in gene expression affected mainly gene transcription and translation, *e.g.*, nucleoside-triphosphatase regulator activity, purine nucleotide binding and double-stranded RNA binding, and signal transduction, *e.g.*, insulin receptor binding, GTPase regulator activity and GTPase activator activity.

Table 2 - Clustering of DEGs based on biological process.

Term	p value
GO:0007422~peripheral nervous system development	5.36E-04
GO:0042491~auditory receptor cell differentiation	0.001356
GO:0046777~protein amino acid autophosphorylation	0.001668
GO:0006412~translation	0.001726
GO:0007242~intracellular signaling cascade	0.001968
GO:0032386~regulation of intracellular transport	0.002223
GO:0008104~protein localization	0.003303
GO:0042490~mechanoreceptor differentiation	0.00344
GO:0046822~regulation of nucleocytoplasmic transport	0.003457
GO:0033157~regulation of intracellular protein transport	0.0035
GO:0010001~glial cell differentiation	0.0035
GO:0006414~translational elongation	0.003612
GO:0007264~small GTPase-mediated signal transduction	0.004034
GO:0007155~cell adhesion	0.004176
GO:0022610~biological adhesion	0.004267
GO:0030534~adult behavior	0.004457
GO:0007243~protein kinase cascade	0.004627
GO:0032387~negative regulation of intracellular transport	0.006022
GO:0010604~positive regulation of macromolecule metabolic process	0.0064
GO:0046823~negative regulation of nucleocytoplasmic transport	0.006632
GO:0016339~calcium-dependent cell-cell adhesion	0.008158
GO:0060117~auditory receptor cell development	0.008276
GO:0043933~macromolecular complex subunit organization	0.008799
GO:0006461~protein complex assembly	0.009174
GO:0070271~protein complex biogenesis	0.009174
GO:0019226~transmission of nerve impulse	0.009828

Altered biological pathways in bladder carcinoma cells

The gene expression profile changed significantly during the progression of bladder carcinoma. Some genes changed significantly under pathological conditions. These DEGs were selected for KEGG pathway enrichment analysis and subsequent identification of the altered pathways in bladder carcinoma cells. Based on a threshold value of $p < 0.1$ and a gene count > 2 we identified eight dysregulated pathways (Table 4). The most significant enrichment involved the complement and coagulation cascades ($p = 0.002326$). Some of the significant pathways were related to signaling transduction, such as PPAR signaling pathway ($p = 0.052499$) and SNARE interactions in vesicular transport ($p = 0.075573$).

Identification of related active small molecules

The DEGs were classified into up-regulated and down-regulated gene groups followed by GSEA analysis and matching after small molecule treatment in the CMap

Table 3 - Clustering of DEGs based on molecular function.

Term	p value
GO:0005158~insulin receptor binding	2.47E-05
GO:0060589~nucleoside-triphosphatase regulator activity	7.39E-04
GO:0030695~GTPase regulator activity	0.001494
GO:0017076~purine nucleotide binding	0.002422
GO:0003725~double-stranded RNA binding	0.002958
GO:0005096~GTPase activator activity	0.004455
GO:0003735~structural constituent of ribosome	0.005429
GO:0000287~magnesium ion binding	0.006179
GO:0051219~phosphoprotein binding	0.006982
GO:0022843~voltage-gated cation channel activity	0.007117
GO:0032553~ribonucleotide binding	0.007602
GO:0032555~purine ribonucleotide binding	0.007602
GO:0031420~alkali metal ion binding	0.008589

Table 4 - Altered biological pathways in bladder carcinoma cells.

Term	p value
hsa04610:Complement and coagulation cascades	0.002326
hsa05020:Prion diseases	0.018793
hsa05414:Dilated cardiomyopathy	0.028013
hsa03010:Ribosome	0.030355
hsa04914:Progesterone-mediated oocyte maturation	0.050126
hsa03320:PPAR signaling pathway	0.052499
hsa04130:SNARE interactions in vesicular transport	0.075573
hsa04270:Vascular smooth muscle contraction	0.097736

database. This analysis identified some small molecules that could reverse the status of bladder carcinoma cells. Table 5 shows the 20 most significant small molecules identified by this procedure.

Thioguanosine (enrichment = 0.865) and tyloxapol (enrichment = 0.857) can partially imitate the carcinoma status of bladder cells, *i.e.*, these small molecules may be strong inducers of bladder cancer. In contrast, adiphenine (enrichment = -0.962) and viomycin (enrichment = -0.947) can imitate the status of normal cells, *i.e.*, they can reverse the abnormal status and are therefore potentially lead molecules for developing new therapeutic drugs for treating bladder carcinoma. Further investigation of these small molecules may clarify their role in the pathogenesis of bladder cancer.

Table 5 - List of small molecules that can imitate the status of normal cells.

CMap name	Enrichment	p value
Adiphenine	-0.962	0
Viomycin	-0.947	0
Trichostatin A	0.253	0
Isoflupredone	-0.969	0.00008
Fludrocortisone	-0.693	0.00022
Biperiden	-0.845	0.00026
Thiamphenicol	-0.826	0.00040
Thioguanosine	0.865	0.00046
Finasteride	-0.752	0.00046
Prestwick-692	-0.882	0.00048
Genistein	-0.471	0.00048
Gentamicin	-0.875	0.00052
Tyloxapol	0.857	0.00056
Phthalylsulfathiazole	0.813	0.00056
Calcium folinate	-0.811	0.00056
Monensin	-0.73	0.00073
Trimethobenzamide	-0.795	0.00074
Atractyloside	-0.788	0.00082
Etiocholanolone	-0.725	0.00085
PHA-00745360	-0.649	0.00088

Discussion

Bladder cancer involves the proliferation of malignant intrabladder cells and is the fourth most common solid tumor in men and the seventh most common in women worldwide, with 350,000 new cases each year. The American Cancer Society (AGS) statistics indicate that in 2006 there were 61,420 new cases of bladder cancer and 13,060 deaths. In China, bladder cancer is also commonly associated with urinary tract malignant tumors. The morbidity in 2005 was 4/100 in males and 1.5/100 in females. The steady increase in the morbidity of bladder cancer in some Chinese cities in recent years indicates a need for additional research on this disease.

The main biological processes that showed DEGs based on GO cluster analysis were signal transduction, physical immunity and disease initiation. Nearly 2800 genes were differentially expressed in bladder carcinoma compared with normal cells. These DEGs may be important for investigating the mechanism of disease development and may be useful targets for treating bladder carcinoma, hence the potential interest in studying these genes. The results of GO cluster analysis and pathway enrichment analysis of DEGs suggested that the development of bladder carcinoma may involve important changes in cellular signal transduction.

As a signal molecular receptor, SNARE plays a key role in signal transduction (Skalski *et al.*, 2011). Changes in SNARE interactions in vesicular transport directly affect cellular transduction (Fasshauer, 2003; Han *et al.*, 2009). Peroxisome proliferator-activated receptors (PPAR), which are nuclear hormone receptors (Umemoto and Fujiki, 2012), regulate gene expression and changes in the PPAR signaling pathway result in abnormal gene expression. These signaling pathways regulate cell growth (Sertznig *et al.*, 2008), differentiation and proliferation (Peters *et al.*, 2012) by modulating the expression of downstream genes. The changes mediated by these signaling pathways ultimately affect protein synthesis, such as the ribosomal pathway and development (progesterone-mediated oocyte maturation) (Fauconnet *et al.*, 2002). Such changes are closely related to the onset of bladder carcinoma (Yoshimura *et al.*, 2003).

Based on the DEGs and small molecule interference data we identified a set of small molecules that can imitate the normal cell status, *i.e.*, they can reverse the abnormal gene expression of bladder carcinoma. These small molecules may provide lead compounds for developing new therapies for treating bladder carcinoma. For example, adiphenine inhibits nicotinic receptors, thereby decreasing the frequency of acetylcholine-induced single-channel currents and cluster duration (the latter by 36-fold at 100 μM) while increasing the decay rate (IC_{50} = 15 μM) of macroscopic currents and accelerating desensitization from the open state; however, prior application of the drug to resting

receptors is required for these effects to be seen.(Spitzmaul *et al.*, 2009). The nicotinic receptor is reported to have an important role in various types of cancer (Russo *et al.*, 2012). Hence, adiphenine is a potential therapeutic antagonist for bladder carcinoma. The small molecules that imitate the normal cell status identified in this study may therefore offer new fields of research in bladder cancer therapy.

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