In silico study of gene expression changes in prostate cancer due to endocrine-disrupting chemicals

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Abstract
Prostate cancer is among the frequent causes of malignancy worldwide with incidence rates higher in African-Americans. Endocrine disrupting chemicals are toxicants present in the environment associated with risk of prostate cancer. These chemicals are abundant in the environment like BPA, DDT etc. which have carcinogenic potential. Mechanisms that cause prostate cancer have been predominantly linked to endocrine signaling pathways. While their mechanisms are still being understood, the role of SOX9 as a regulator has been elucidated in prostate cancer.

Gene expression profiles of prostatic cancer available in public databases were compared with metastatised and normal controls to obtain high scoring target genes which were differentially expressed. We compared primary prostate cancer versus metastatic prostate cancer signature profiles and shortlisted genes using GEO2R analysis. The connectivity map database was used to identify compounds which targeted the genes having molecular signatures antagonistic to the target genes. We were successful in identifying many genes which were differentially expressed. We also performed drug repurposing study on the identified genes and located drugs which are in phase 2 or phase 3 or preclinical stage of clinical trials.

Keywords: Endocrine disrupting chemicals, prostate cancer, microarray, drugs.

Introduction

Endocrine disrupting chemicals are exogenous chemicals that impair the endocrine function and are known to cause cancer[3,7,10]. One of the factors that drives malignancy is androgen receptor signalling. The receptor signalling axis drives progression of carcinogenesis[1]. Another critical downstream regulator to ETS transcription factor are SOX9 which is key for development of prostate which was found to be reactivated in prostate cancer. It has been shown that SOX9 activated further pathway genes regulating and causing invasiveness of prostate cancer[4,13,14].

The reactivation of androgen receptor has been reported in resistant cancers of prostate by mechanisms where the androgen receptor gene expression is increased. The major mechanism by which the receptor affects the cells is by transcriptional regulation of gene expression of target genes[9]. Further studies in mice showed that SOX9 also had a role in recurrent cancers of prostate and expressed at higher frequency in castration-resistant tumors. Endocrine disrupting chemicals, however, acting through signaling pathways in prostate cells of adults may transform into tumor cells, though the exact mechanism have not been known.

Microarray technology is a powerful tool for simultaneously assessing the expression of a number of genes and to identify patterns of gene expression associated with a disease. Experiments using microarrays have been deposited in NCBI GEO. Another very useful tool, the Connectivity Map for searching candidate agents as drugs based on gene arrays has been used in cancer research to find strategies for treatment. It has successfully revealed the anticancer activity in lung cancer[5]. Drug repurposing is gaining popularity since it is aimed at finding new and better uses of drugs already in development or clinical use.

Recent years have seen an increase in studies related to transcriptional changes and biomarker discovery caused by endocrine disrupting chemicals[11,14]. The present study is focussed on metaanalysis and looking for possible biomarkers in SOX9 dependent study which would be useful predictor of carcinogenesis of prostate. An additional study on drug repurposing has been conducted based on gene targets identified. The study has significance is understanding gene expression changes causing prostate cancer and identification of drug targets and their inhibitors for repurposing of drugs under clinicians guidance.

Material and Methods

Public Database: NCBI (National Centre for Biotechnology Information) archives datasets of expression data in prostate cancer in GEO profiles. The frozen biopsies were used to isolate tumour samples by laser capture microdissection and microarrays designed according to the protocol[2]. All the available 51 samples of localised and metastatic prostate cancer samples were downloaded from GEO datasets of NCBI. 22 samples belonged to primary localised type, 29 were from metastatic group with 4 controls. The arrays were designed using Affymetrix (human genome).

Connectivity Map database: The Connectivity Map (CMap) database collects gene-expression profiles of human cancer cells, treated with drugs widely used for investigation of drug repurposing. It has a catalogue of cellular signatures hosted by Broad Institute with a genomic library of

transcriptional responses to disease perturbation with more than a million profiles. Data of genetic signatures arising from genetic pertubation through overexpression or gene knockdown or signatures arising from treatment with small compounds for cancer in the database using L1000 mRNA profiling are compared[12]. Various tools and datasets can be accessed at CLUE (Connectivity Map Linked User Environment with web address https://clue.io).

Statistical analysis: GEO2R available at NCBI as online tool was used to perform comparisons on original processed data tables using the GEOquery which was integrated into limma R packages of Bioconductor project. The differentially expressed genes were tabulated using an adjusted p-value by Benjamini and Hochberg (false discovery rate) and logFC > 2 and < -2.

The accesson numbers (GSE32269) of Affymetrix GeneChip Human Genome U133A Array (HG-U133A) were used for downloading and further processing. The top250 hits obtained were processed. The connectivity map database was accessed for all the gene differentially expressed using the above criteria. The data were classified based on mechanism of action (MOA) and gene ontology (GO).

Results and Discussion

Table 1 shows a list of genes which are overexpressed where androgen receptor-mediated regulation was studied in human tissue and the role of SOX9 mediated transcription was studied. Kallikrein related peptidase 3 (KLK3) is overexpressed by 150 fold. Keratin 18 (KRT18), SAM pointed domain containing ETS transcription factor, ERG-ETS transcription factor, glutathione S-transferase zeta 1(GSTZ1) and microtubule associated protein 7 (MAP7) which are expressed 70 to 150 fold.

A total of 36 differentially expressed genes were extracted from the top 250 hits of GEO2R analysis using MOA criteria from the gene list in the Cmap database. Out of these, 6 were new drugs with potential to be used as gene targets in prostate cancer. The top hits were kallikrein related peptidase 3 (KLK3), Rho GTPase activating protein 25 (ARHGAP25), keratin 18 (KRT18), NK3 homeobox 1(NKX-1) with gene ontologies listed (Table1). The drugs which are at the phase 2 of drug discovery are 4-CMTB, 2,5-furandimethanol, isobutyramide, ibrolipim with gene targets being free fatty acid receptor agonist (FAR2), hemoglobin modulator (HBB), gene expression stimulant (HBG1) and lipoprotein lipase activator(LPL) respectively. The drugs dextromethorphan and gemfibrozil are being launched for treatment in other areas (Table 2).

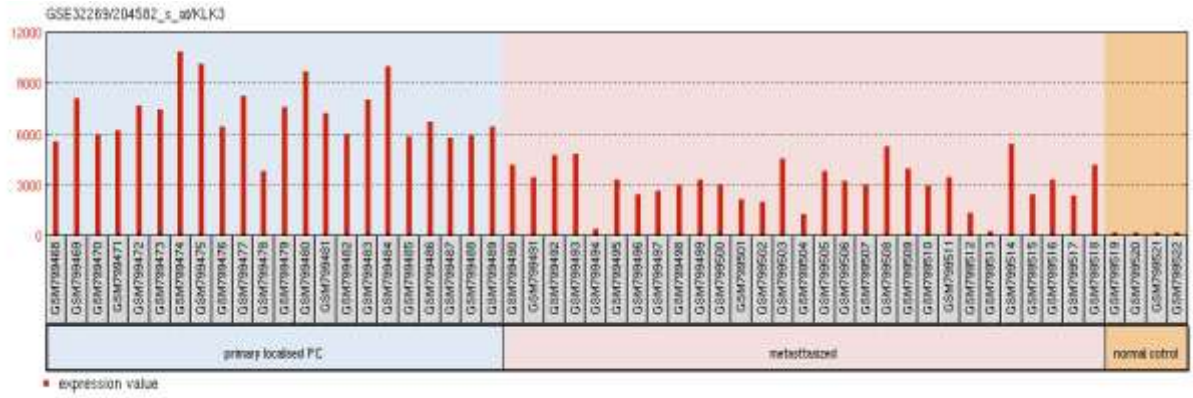


Fig. 1: KLK3 gene which are overexpressed as compared to normal control.

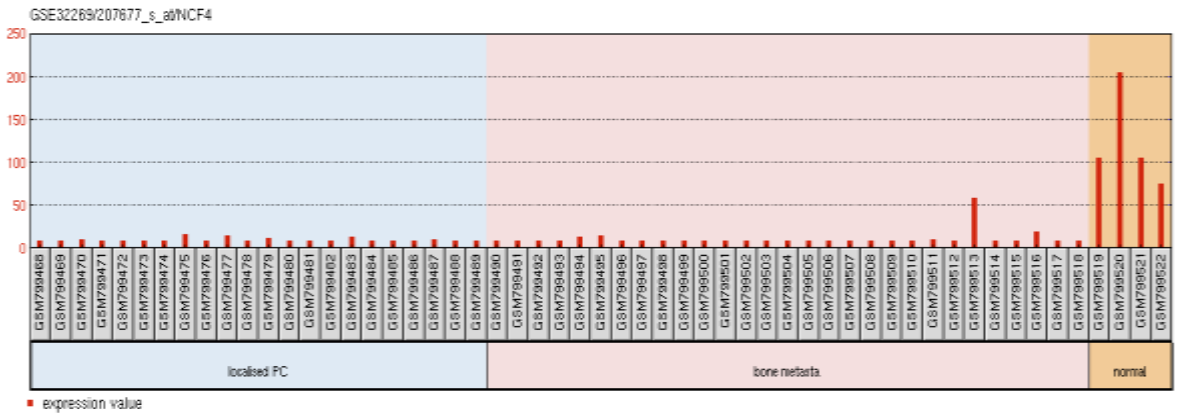


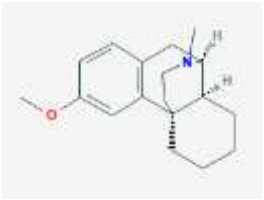
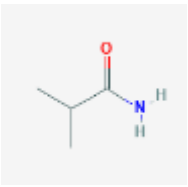
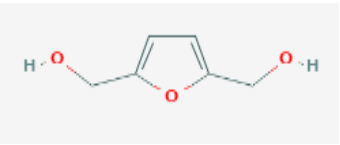
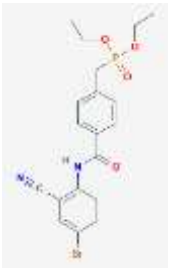
Fig. 2: Under expression of neutrophil cytosolic factor 4 (NCF4) in primary localised and bone metastasis samples compared with normal bone.

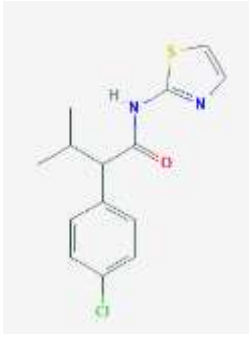
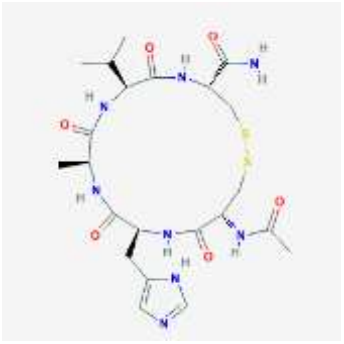
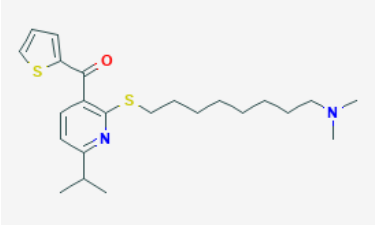
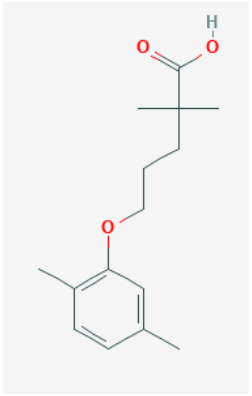
The negative expression of NCF4 in primary localised and bone metastasis samples in GEO database has a corresponding drug acting as sigma receptor agonist. NCF4 which is a gene involved in innate immunity associated with colorectal cancer[8]. In the present work NCF4 has been proposed to have a role in prostate cancer. The Cmap database identified the drug dextromethorphan as sigma receptor agonist (table 1). The drug dextromethorphan binds to sigma-1 (σ1) receptors and is an effective antitussive having antidepressant effects[6].

Table 1
List of gene targets differentially expressed categorised using Gene ontology analysis

S.N.	Gene symbol	Gene name	Fold expressed	GO function
1	KLK3	kallikrein related peptidase 3	271	Endopeptidase activity
2	ARGHAP25	GTPase activator	210	Rho GTPase activating protein 25
3	KRT18	Keratin 18	163	Cadherin binding
4	NKX3-1	NK3 homeobox-1	159	MADS box domain
5	LST1	leukocyte specific transcript 1	133	Leukocyte specific transcript
6	SLC39A6	solute carrier family 22 member 4	133	Zincion transmembrane transporter activity
7	CNN1	Calponin1	130	Actin binding
8	HBB	Haemoglobin subunit	130	Haptoglobin binding
9	MYH11	Myosin heavy chain	124	ATP binding

Table 2
Drug repurposing for the gene target listed with respective stage of clinical trials/phases

S.N.	Drug	Gene target	Drug structure	Stage of clinical trial
1.	Dextromethorphan (sigma receptor agonist)	Neutrophil cytosolic factor 4 (NCF4)		Launched as Antitussive
2.	Isobutyramide (gene expression stimulant)	HBG1		Phase 2
3	2,5-furandimethanol (hemoglobin modulator)	HBB		Phase 2
4	Ibrolipim	LPL activator		Phase 2

5	4-CMTB	Fatty acid receptor agonist (FAR2)		
6	Exherin	Cadherin (CDH2)		Phase 2
7	S-17092	Endopeptidase inhibitor		preclinical
8	Y-29794	Endopeptidase inhibitor		preclinical
9	Gemfibrozil	LPL activator		Launched, for treating abnormal lipid levels

Conclusion

Endocrine disrupting chemicals which are commonly present in the environment have deleterious effects like causing prostate cancer. The Sox9 regulation has been involved in initiation of prostatic cancer. In the meta-analysis, Sox9 based prostate cancer data have been analysed to look at genes which are showing changes in gene expression.

With the availability of new drugs constantly being launched for clinical trials, it is vital to perform metaanalysis of deposited data and look for new targets for treatment of prostate cancer both localised and metastatic. The drug target NCF4 showed binding to drug dextromethorphan, an antitussive as well as gemfibrozil used for treating abnormal lipid levels binding LPL activator. Such repurposing of drugs generated from databases could lead to fewer side effects as well discovering targets for further study. The present work would lead to additional strategies to control prostate cancer with phase 2 drugs as soon as it is launched and could help clinicians in leading to faster regression of cancer.

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