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RESEARCH ARTICLE

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Drug repurposing analysis with co-expressed genes identifies novel drugs and small molecules for bladder cancer

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Abstract

Bladder cancer (BC) is the fifth most common malignancy in humans and has poor survival rates. Although there is extensive research on the diagnosis and treatment of BC, novel molecular therapies are essential due to tumor recurrence. In this study, we aim to identify repurposed drugs or small molecules of BC with multi-omics systems biology perspective. Gene expression datasets were statistically analyzed by comparing bladder tumor and normal bladder tissues and differentially expressed genes (DEGs) were determined. Co-expression network of common DEGs for BC was constructed and co-expressed module was found by using tumors and control bladder tissues. Using independent data, we demonstrated the high prognostic capacity of the module genes. Moreover, repurposed drugs or small molecules were predicted by using L1000CDS2 gene expression based-search engine tool. We found numerous drug candidates as 480743.cdx, MK-2206, Geldanamycin, PIK-90, BRD-K50387473 (XMD8-92), BRD-K96144918 (mead acid), Vorinostat, PLX-4720, Entinostat, BIX-01294, PD-0325901 and Selumetinib, that may be used in BC therapy. We report 480743.cdx, BRD-K50387473 (XMD8-92) and mead acid as novel drugs or small molecules that offer crucial step in translational cancer research of BC.

Keywords: multi-omics data, gene expression, drug repurposing, bladder cancer.

1. Introduction

Bladder cancer (BC) is the fifth most common malignancy in humans and approximately 550,000 new cases occur per year with 200,000 of them resulting in death [1,2]. Approximately, 75% of the tumors do not invase to muscularis propria and these are classified as non-muscle invasive bladder cancer (NMIBC)[3]. Muscle invasive bladder cancer (MIBC) is associated with most bladder cancer morbidity and mortality. Early-stage cancers are mostly treated with tumor resection, but the disease recurrence rate is high (50-80%) and can progress to an invasive type depending on the stage. Therefore, patients usually undergo lifelong surveillance through cystoscopy [4,5].

Chemotherapy and radical cystectomy are the routine treatment methods and immunotherapies are in the clinical trial phase. Also, bladder cancer is a curable disease, therapy is a difficult process and MIBC has a poor survival rate

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of 5-years <50%, but if the tumor spreads to lymph nodes or organs, the survival rate decreases to 35% [3,6]. So, early diagnosis is of vital importance.

Diagnosis of bladder cancer is practiced by standard tests like urine cytology, but the sensitivity is as low as 40% [7]. Multiplex tests including different markers will probably provide higher precision. In recent years, high-throughput molecular profiling studies identified novel biomarkers and therapeutic targets for different cancers [8]. Bladder cancers can be diagnosed without muscle invasion through molecular screening by the use of tumor biomarkers. Thus, metastase- induced morbidity can be prevented and life expectancy may be improved.

The advent of molecular biology methods positively affects the diagnosis and the prediction of outcomes of several cancers. Detailed multi-omic studies are carried out for the discovery of carcinogenesis and progression. In bladder tumors MIBC and NMIBC show different molecular characteristics and their clinical behaviors are distinct [9]. Due to the complexity and heterogeneous structure of bladder cancer, multiple biomarkers are investigated concurrently and some of them accurately predict the prognosis [10]. Multi-omic studies are crucial at this point considering genomic aberrations in tumor transformations. There are few studies focused on bladder cancer, and NMIBC- related research is more limited. Goel et al. used exome and transcriptome sequencing to characterise all grades of NMIBCs to determine prognostic genes and indicated that multi-omic data may help to better identify treatment in high-risk patients [11].

Although there is extensive research on diagnosis and treatment of BC, new molecular therapies are required due to tumor recurrence. Drug repurposing is aimed at approved or failed/abandoned compounds to find new indications for use in a different disease or condition. Drug repurposing studies offer an alternative to conventional drug inventions with their cost effective, cheaper and time saving aspects. For the development and release to the market of a new drug molecule, an average of 12-13 years and an estimated 2-3 billion USD investment are required. Also, the proposed drug is safe as it has been approved by a health regulatory authority. For cancer treatment, there are three repurposed drugs [12,13]. Feng et al. investigated metformin for BC therapy. Metformin is a frequently used hypoglycemic drug and it has been reported in the study that metformin has an anti-proliferative effect on BC stem cells and support the chemotherapy drugs on BC cells [14].

Developing potential marker gene lists of bladder cancer must be the starting point. Lindskrog et al. described transcriptomic and genomic markers of NMIBC and presented an online classification tool [4]. In a whole exome sequencing study, driver mutations in FGFR3, KDMTA, and KDMT2C were found and also DNA methylation and hydroxymethylation were investigated as promising biomarker [5]. Also, it was indicated in the literature that CCNB1, FOXM1, GSN, LAMC2 genes are prognostic expression markers for non-invasive BC [7]. Besides, there are some studies about identifying key genes and pathways in bladder cancer. Gao et al. showed in their GO analysis that mitotic nuclear division, the spindle and protein binding related genes upregulated while cell adhesion, extracellular exosomes and calcium ion binding related genes downregulated [15]. In another research, differentially expressed genes in bladder cancer tissues were identified as mitotic and chromosome assembly, including nucleosome assembly, spindle checkpoint and DNA replication [16]. Also, Tang et al. reported that upregulated DEGs were associated with cell division, nucleoplasm and protein binding, while the downregulated DEGs were associated with ‘extracellular matrix organization’, ‘proteinaceous extracellular matrix’ and ‘heparin binding’ [17].

In the present study, differentially expressed genes (DEGs) were identified by using gene expression datasets including bladder tumor and normal bladder tissues obtained from two different studies. BC specific co-expression network of common DEGs was reconstructed. A co-expressed module was found by using cancerous and normal bladder tissues. The prognostic capability of the module was evaluated. Moreover, potential therapeutic targets and reverse the expression of co-expressed module genes were investigated through L1000CDS2 tool. We report novel

drugs or small molecules that offer crucial prospects for prognosis, treatment and translational cancer research of bladder cancer.

2. Materials and methods

2.1. Transcriptome datasets

The data of transcriptome datasets of BC including GSE7476 [18] and GSE24152 [19] were taken from Gene Expression Omnibus [20]. It was analyzed to identify differentially expressed genes (DEGs) of BC. Both datasets including the arrays of the Affymetrix platform were selected for analysis. A total of 27 samples were selected, including 17 BC and 10 normal bladder tissue samples. BLCA-TCGA-Bladder Urothelial Carcinoma obtained from the Cancer Genome Atlas (TCGA) database as an independent dataset including 390 patients was used in prognosis analysis.

2.2. Identification of differentially expressed genes

Robust Multi Array (RMA) techniques [21] were used for normalization of datasets. Linear models for microarray (LIMMA) package [22] in R language were performed for both dataset to identify DEGs in patients with BC compared to healthy individuals. The obtained DEGs were determined based on the p-values ($p < .05$) and the direction of differentiation was identified using gene expression fold changes (FC). Up regulated and down-regulated genes were identified considering the FC> 2 and FC <0.5, respectively.

Gene enrichment analyses of DEGs were performed via the Metascape [23]. The significant terms were determined by using $p < 0.05$ which is the cut-off for statistical significance.

2.3. Differential co-expression analysis and identification of co-expressed modules

Gene expression data of common DEGs of two datasets were obtained from both tumor and control samples, separately. Our differential co-expression network analysis algorithm [24] was applied to both gene expression data of cancerous and normal tissues to identify a BC specific differentially co-expressed network. The mean value of gene expression data of each common DEG was calculated. Afterward, z score normalization of each common DEGs was found. Spearman correlation coefficients (SCC) of mean gene expression were calculated in BC and normal bladder tissues, separately since data are not normally distributed. The significant pair-wise gene correlations of common DEGs were determined by using an SCC cutoff ($p < 0.05$). It was constructed a BC specific differential co-expression network in cancerous samples compared to normal bladder tissues. Two parameters were described to identify significant differentially co- expression profiles between cancerous and normal tissues: (i) Gene pair that show a significant correlation score in the cancerous samples, but no significant correlation in the normal bladder samples. (ii) Although gene pair show a significant correlation in both cancerous and normal bladder samples, it was selected co-expression direction is different in cancerous and normal bladder samples (i.e: positive and negative correlation score).

The MCODE plugin [25] of the Cytoscape [26] was used to identify network modules of the differential co-expression network. For further analysis, modules with a minimum of 10 nodes (genes) and a network density of 0.50 were taken into consideration.

2.4. Prognostic capability analysis of co-expressed module genes

An independent BC dataset obtained from TCGA was used to investigate the prognostic capabilities of the co-expressed module genes. Cox proportional hazards regression analysis was executed via SurvExpress bioinformatics

validation tool [27]. In SurvExpress, each cancerous sample of the BLCA-TCGA-Bladder Urothelial Carcinoma was categorized according to their prognostic index as low- and high-risk groups. The prognostic performance of the module genes was determined through the log-rank test and Kaplan-Meier (KM) plots.

2.5. Drug repurposing analysis

L1000CDS2 [28] is a search tool that presents a listing of FDA-approved drugs or experimentally studied small molecules that are defined to reverse or mimic the down-regulated and up-regulated genes. It was executed L1000CDS2 analyses by using the differential co-expressed up and down regulated module genes, to identify drug or small molecules that reverse the regulation direction of genes in BC (https://maayanlab.cloud/L1000CDS2/#/index).

3. Results

3.1. Gene expression profiles of bladder cancer

Differentially expressed genes (DEGs) for BC were identified in BC compared to normal bladder samples through statistical analysis. We obtained 2490 DEGs (p value<0.05) where 727 upregulated and 1769 downregulated genes from GSE7475 datasets. Analysis of GSE24152 identified 832 DEGs (p value <0.05), 483 upregulated and 725 downregulated DEGs. (Figure 1A). 131 common DEGs between the two datasets were determined (Figure 1B). The gene enrichment analysis of common DEGs indicated that signaling by aberrant PI3K in cancer, ras signaling, cytoskeleton and proteoglycan-related biological processes were significantly enriched terms (Figure 1C).

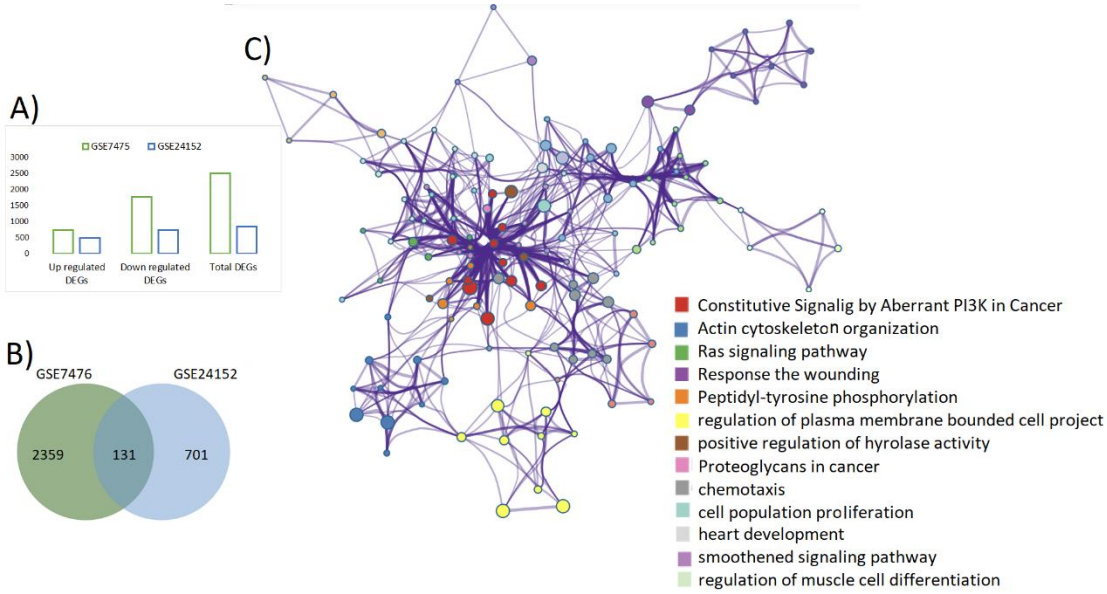

Fig. 1. Gene expression analysis results of bladder cancer. (A) The graph of up-regulated genes, down-regulated genes and total differentially expressed genes (DEGs) (p <.05). (B) The venn diagram represents the number of common DEGs between both datasets. (C) Biological pathway and gene ontology enrichment analysis results of common DEGs. The network was obtained from Metascape bioinformatics tool.

3.2. Differential co-expression network in bladder cancer

The differential co-expression network analyses resulted in a total of 759 significant differential correlations among 131 common DEGs in cancerous tissues compared with normal bladder tissues. Differentially co-expressed gene module which is highly-clustered co-expressed genes including the number of 22 nodes (ETV4, SLC44A5, EVPL, ARL13B, COPZ1, EPRS, ELN, EPHA3, GLTP, SSBP2, SLC39A11, MSN, SCRIB, FGFR1, MLXIP, EMP1, EPHA7, FAM83B, JAZF1 , CCT5, FGFR3 and EPB41L2) and 191 edges and a network density is 83% was obtained (Figure 2). It was performed gene enrichment analysis on the module genes. The statistically significant biological process associated GO terms (p value <0.05) were obtained, Top three terms were identified as transmembrane receptor protein tyrosine kinase signaling pathway (GO:0007169), cell recognition (GO:0008037) and morphogenesis of an epithelium (GO:0002009) (Figure 2).

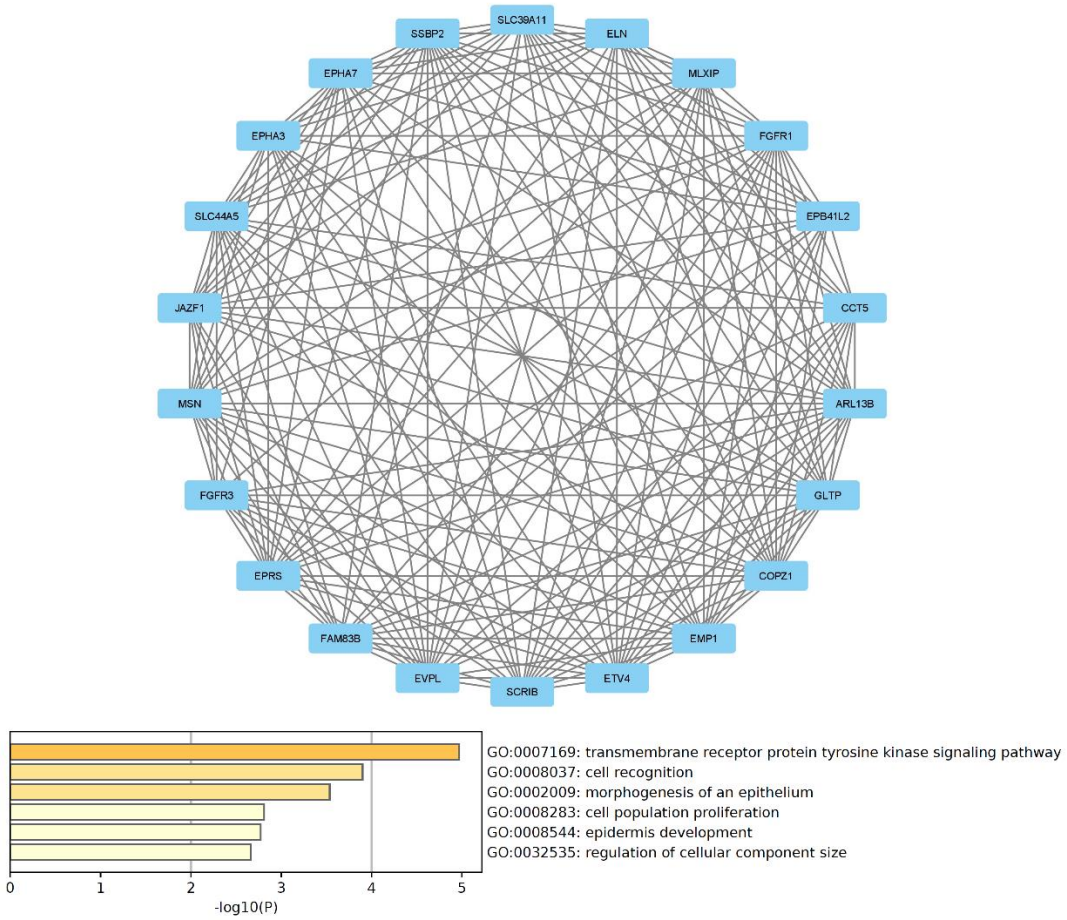

Fig. 2. Differential co-expressed module in bladder cancer. Statistically significant differentially correlated common DEGs were represented as nodes and significant Spearman correlation values between the DEGs were represented as edges.

3.3. Prognostic capability of module genes by using independent bladder cancer dataset

To determine the prognostic capability of the module genes, Cox proportional hazards regression analysis was

executed in SurvExpress validation bioinformatics tool. Cancerous tissue samples were categorized into low- and high-risk groups according to their prognostic index calculated by using survival times. For this purpose, an independent RNA-Seq dataset (n = 390), BLCA-TCGA-Bladder Urothelial Carcinoma-July 2016, was performed and prognostic capabilities of the module genes based on survival data were analyzed by using the log-rank test and Kaplan-Meier plots ($p<0.01$) (Figure 3).

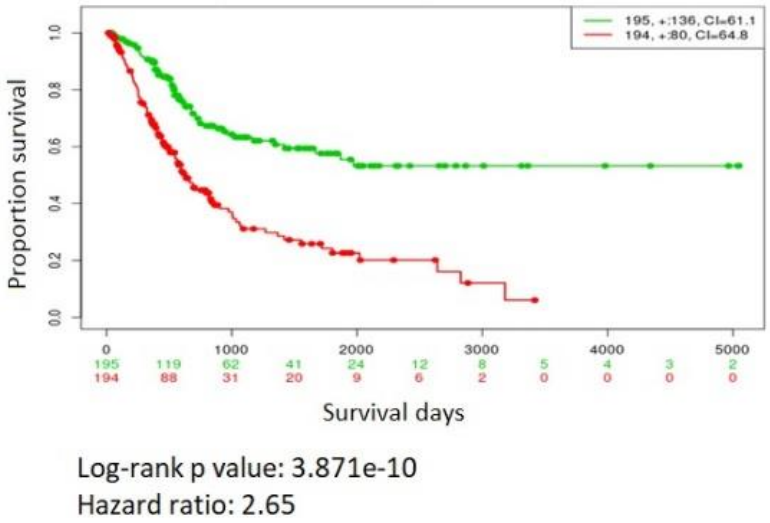

Fig. 3. The survival analysis results of the BC specific module genes were represented with Kaplan-Meier curve. Low-risk and high-risk of patients samples were represented as green and red colors, respectively.

3.4. Putative repurposed drugs and small molecules for bladder cancer

The LINCSL1000 gene expression based-search engine tool was utilized concerning to the up and down-regulated module genes as predictive molecular targets in BC. The L1000CDS2 web tool was used to enter the list of up and down regulated module genes in order to search for drugs or small compounds that could change the gene expression profiles of the relevant genes. With an overlap score of ≥ 0.1765, the highest score to reverse expression profiles on up-regulated and/or down-regulated module genes in various cell lines, thirteen drugs or small compounds were obtained as repurposed drugs, demonstrating potential (Table 1). Repurposed drug or small molecule candidates were investigated in the literature to understand whether BC is associated with or not.

Table 1. Repurposed drugs and small molecules for bladder cancer with an overlap score of ≥ 0.1765 according to LINCS L1000 gene expression based-search engine tool analysis.

Rank	Score	Perturbation	Cell line	Dose, µm	Time, h	Reversed expression of genes
1	0.1765	480743.cdx	HT29	80.0	24.0	*EPB41L2,EPHA3,FGF1*
2	0.1765	MK-2206	LOVO	10.0	6.0	*EMP1, FGFR1, MSN*
3	0.1765	Geldanamycin	NCIH2073	10.0	6.0	*EMP1, ELN, EPHA3*
4	0.1765	PIK-90 (PI 3-K inhibitor IX)	RMGI	10.0	6.0	*EMP1, FGFR1, MSN*
5	0.1765	BRD-K50387473(XMD8-92)	HEPG2	10.0	6.0	*EMP1, FGFR3, GLTP*
6	0.1765	BRD-K96144918(Mead acid)	A549	10.0	6.0	*EMP1, FGFR3, SSBP2*
7	0.1765	Vorinostat(SAHA,suberoylanilide	MCF7	10.0	6.0	*EPB41L2, EPHA3, MSN*

		hydroxamic acid)				
8	0.1765	PLX-4720	A375	1.11	24	*ETV4, EPHA3, SSBP2*
9	0.1765	PLX-4720	A375	0.37	24	*ETV4, EPHA3, SSBP2*
10	0.1765	Entinostat (BRD-K77908580)	MCF7	3.33	24	*EPRS, FGFR1, MSN*
11	0.1765	BIX-01294	HEPG2	10	24	*FGFR3, FGFR1, MSN*
12	0.1765	PD-0325901	A375	0.04	24	*ETV4, EPHA3, SSBP2*
13	0.1765	Selumetinib	HME1	10	24	*EMP1, ETV4, SSBP2*

4. Discussion

Bladder cancer (BC) is among the top 10 most common tumors with the 6th most diagnosed cancer worldwide [14]. Pathologically, BC is categorized as NMIBC and MIBC. Due to its clinical and molecular complexity, it is not possible to forecast which stage tumor will progress to an aggressive form. In our study, we found thirteen drugs or small molecules that can reverse gene expressions and searched for BC. There are few genomic aberrations in FGFR3, PIK3CA, RAS oncogenes on bladder cancer and these genes targeted treatments are being investigated in several clinical trials.

Also, there are essential molecular pathways that act in urothelial tumorigenesis as the RAS-MAPK pathway and PI3K-Akt pathway [29]. MK2206 is an allosteric Akt inhibitor, that blocks the phosphorylation and activation of Akt, therefore prevent the proliferation of many human cancer cell lines [30]. There are several searches on the effect of MK2206 on breast, thymic, lung, colorectal, endometrial, renal cancers [31-34]. Sathe et al. examined MK2206 on 11 BC cell lines and stated a decreasing AKT phosphorylation depending on the dose. They also specified that an increase in caspase 3/7 activity of sensitive cells is related with an increase in apoptosis [31]. In another study Sun et al. showed the booster effect of MK2206 on cisplatin (CDDP)-induced cytotoxicity and bladder cancer cells apoptosis and suppressive effect on tumor growth in subcutaneous xenograft models [35]. They also have seen the same incidence of testicular cancer and elucidated the mechanism by the suppressed expression of Akt pathway [36]. Zhang et al. studied on drug sensitivity and remarked that bladder tumor cells with Retinoblastoma 1 (RB1, a cell division regulator) mutation (found in 25% of patients) are more resistant to MK-2206, Dactolisib and GNE-317 [6].

The second drug candidate is geldanamycin, a type of antibiotic from the benzoquinone ansamycins (BAs) family. It has heat shock protein 90 (Hsp90) inhibitory properties. Hsp90 upregulation in malignancies acts as protective for tumorigenesis, therefore Hsp90 inhibition is one of the trend cancer treatment modalities. Geldanamycin has been used in numerous cancer studies as gall bladder, thyroid, liver, osteosarcoma, lungs, melanoma, cervical, breast, prostate, colorectal cancers [37-40]. Germano et al. emphasized that Ron can gain tumorigenic potential by single point mutations and aberrant activation of Ron has been determined in colorectal adenocarcinomas, non-small cell lung tumors and primary breast carcinomas. Also, it is stated that Ron expression is correlated with bladder tumor phase [41]. Karkoulis et al., demonstrated anti-neoplastic properties of geldanamycin in human bladder tumor cell lines (RT4 and T24) [42]. Unfortunately, it is instable, cardiotoxic, oculotoxic, hepatotoxic and has low aqueous dissolubility, therefore several GA analogues have been produced [39,43].

Re-regulation of the phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway in tumors is also one of the most researched cancer therapies. PIK90 is a small molecule that acts as a PI3K inhibitor and its antitumor activity has been shown for breast, ovarian cancer cells [44] and bladder cells [45]. In metastatic bladder cancers, the PI3K pathway is around 72% overactive. Sathe et al. characterized molecular mechanisms of PI3K pathway signaling in bladder cancer cells in their study. They used different pathway inhibitors and small molecules such as PIK90 and MK-2206 and demonstrated their anti-cancer properties. Finally, they emphasized that

simultaneous targeting of the PI3K, AKT and mTORC1 pathways is required for effective tumor growth inhibition [46].

Histone deacetylases regulate the expression of numerous proteins involved in malignant tumor initiation and progression. Therefore, histone deacetylase inhibitors (HDACi) are being produced for cancer treatment. Vorinostat is one of them and approved by the U.S. Food and Drug Administration (FDA) for the cure of advanced and refractory cutaneous T-cell lymphoma. It has been also applied in numerous clinical cancer therapy trials such as head and neck squamous cell carcinomas, breast, lymphoma, non-small cell lung cancer (NSCLC), glioblastoma multiforme [47]. Besides, vorinostat reportedly has antiproliferative effects in various cancer cells such as ovarian cancer cells [48], renal cancer cells (in combination with Fluvastatin [49]), breast cancer [50]. and human bladder cancer cell lines [51]. Kaletsch et al. examined the effect of a novel HDACi 19i (LMK235) with vorinostat (SAHA) and the HDAC4-specific HDACi TMP269 on urothelial carcinoma cells, and stated the disturbed mitosis with apoptosis of cells after treatment [52]. In a clinical study, Quinn et al. observed the toxic effect of Vorinostat and proposed to use a lower dose [53]. Additionally, vorinostat has low solubility and permeability. Moreover, due to its high metabolized in the liver, its therapeutic benefits are poor when used as monotherapy. So various delivery systems are being developed to increase its clinical utility [47].

Nearly 20% of urothelial tumors were identified as RAF1 activation dependent and they use RAF/MEK/ERK signaling pathway. Therefore, these cells are sensitive to RAF inhibitors and RAF plus MEK inhibition combination. Bekele et al. specified in their investigation that bladder cancer cell lines are sensible to BRAFV600E inhibitor PLX4720 [54]. Also, Chen et al., determined Transient receptor potential (TRP) family gene expression in bladder and para-carcinoma tissues and TRP expression is associated with the sensibility to several drugs inclusive of PLX-4720 [55]. Otherwise, PLX4720 is applied for other cancer types such as melanoma [56,57] and colorectal carcinoma [58].

Entinostat is also a selective HDAC inhibitor like vorinostat, and has been investigated as either a single agent or a combination in non-small cell carcinoma, Hodgkin's lymphoma, breast, and myelodysplastic syndrome. Pili et al., used entinostat and 13-cis retinoic acid on patients having solid tumours and searched for its safety [59]. Truong et al., evaluated the anticancer activity and cell-autonomous mechanism of entinostat in bladder cancer and they proved that entinostat had substantial antitumor efficiency in immune-competent but not immune-compromised hosts. Also, they indicated that when entinostat was combined with programmed cell death protein 1 (PD-1), its antitumor responses were increased, and long-term immunologic memory was induced in host [60]. In another study, entinostat was treated with a combination of the approved drug decitabine on platinum resistant bladder tumor cells and researchers proposed its usage on cisplatin-resistive bladder cancer [61]. Additionally, macrophages play an essential role in immune response and tumor-associated macrophages partake in solid tumor development with anti-tumorigenic or pro-tumorigenic character based on their polarization. So, there are various clinical trials targeting macrophages. In bladder cancer research different agents were used as Vorinostat and Entinostat [62].

BIX-01294 is the first selective G9a, a histone methyltransferase also known as euchromatic histone-lysine N-methyltransferase 2 (EHMT2), inhibitor. G9a is highly active in some cancer types as esophageal, ovarian, and gastric cancer. Inhibition of this gene prevents tumor cell proliferation and metastases by stimulating autophagic cell death, apoptosis, and cell cycle arrest. Also, antiapoptotic proteins are decreased and proapoptotic proteins are increased with G9a depletion. BIX-01294 was proven to induce cell death in breast cancer, head and neck squamous cell carcinoma, neuroblastoma cells and bladder cancer cells [63-65]. Cui et al., showed BIX-01294 induced endoplasmic reticulum stress and apoptosis in human bladder cancer cells occurred via caspase-dependent pathway [63]. Li et al. also examined the anti-proliferative effect of BIX-01294 on bladder carcinoma cell lines T24 and UMUC-3 and proposed that G9a might be a good therapeutic target in bladder cancer [66]. In a clinical study, the role of G9a in Bacillus Calmette-Guerin (BCG)-treated NMIBC patients was investigated. BCG bladder instillation is the gold standard treatment in high-risk NMIBC patients. In the experiments BIX-01294 was used to examine the

effect on trained immunity responses in vitro and finally it was emphasized that suppression of G9a is important in the stimulation of trained immunity [67].

Cirone et al. investigated the effect of PI3K/mTOR inhibitor PF-0469502 and a MEK inhibitor PD-0325901 in in vitro and in vivo models of bladder cancer and determined the slowed tumor growth. Therefore, they suggested the therapy as a potential treatment approach for bladder cancer [68]. Also, in an in vivo study, bladder cancer invasion was prevented by TGFβ receptor inhibitor LY2157299 and MEK inhibitor PD-0325901 [69]. Zhang et al., investigated the effect of PD0325901 (MEK/ERK inhibitor), CHIR99021 (GSK3 pathway inhibitor), small molecule inhibitors SB431542 (ALK inhibitor) and valproic acid (VPA; HDAC inhibitor) on uterine cervix carcinoma cells, bladder cancer cells and squamous cell carcinoma cells. The results of the analysis reveal that combined inhibition of MEK/ERK, ALK and GSK3 may be a potential cancer therapy [70].

Selumetinib is a small molecule, having a short half-life, and acts as an oral mitogen-activated ERK kinase (MEK)-inhibitor. There are lots of research about its usage in various cancers such as melanoma, colorectal, pancreatic, breast cancers, papillary thyroid carcinoma, non-small cell lung cancer, pediatric low-grade gliomas and neurofibromatosis 1 (NF1) [71]. Preclinical studies suggest that it may improve the effect of chemotherapeutic drugs. LoRusso et al. demonstrated that selumetinib was safe when combined with docetaxel and dacarbazine in advanced solid tumors [72]. Schulz et al. used gamma-secretase inhibitor (GSI) dibenzazepine and selumetinib in bladder cancer cell line and suggested inhibition of both NOTCH and MAPK signaling most strongly suppressed tumor growth [73]. Additionally, there is an ongoing clinical trial using Selumetinib in Muscle Invasive Bladder Cancer (NCT02546661)

In cancer related studies it was indicated that mead acid containing diet inhibited breast cancer by suppressing cell proliferation, also mead acid inhibited some tumorigenic features of human breast, urothelium, and colon cell lines [74-76].

BRD-K50387473 (XMD8-92) is an extracellular signal-regulated kinase 5 (ERK5), a member of the mitogen-activated protein kinase (MAPK) family, inhibitor. Kang et al., induced apoptosis of acute myeloid leukemia cell lines and they proposed that XMD8-92 may be an efficient adjuvant in AML chemotherapy [77]. Besides, XMD8-92 was utilized in hepatocellular carcinoma cells [78]., lung and cervical cancers, pancreatic cancers [79]. and Yang et al., emphasized that XMD8-92 may be an effective approach for treating human cancer [80].

In summary, in our study bladder cancer associated genes were found to be associated with transmembrane receptor protein tyrosine kinase signaling pathway, cell recognition and morphogenesis of an epithelium. We found numerous drug candidates as 480743.cdx, MK-2206, Geldanamycin, PIK-90, BRD-K50387473 (XMD8-92), BRD-K96144918 (mead acid), Vorinostat, PLX-4720, Entinostat, BIX-01294, PD-0325901 and Selumetinib, that may be used in bladder cancer therapy. As discussed above, all candidates are investigated in terms of bladder cancer except from 480743.cdx, BRD-K50387473 (XMD8-92) and mead acid. These three prospective drugs may be evaluated for bladder cancer therapy after carrying out more advanced analyses and preclinical studies.

Acknowledgement

All authors declared that there are no conflicts of interest.

Appendix

Availability of data and materials

Transcriptomic data have been deposited in Gene Expression Omnibus with an accession number GSE7476 and GSE24152. For validation studies, it was used BLCA-TCGA-Bladder Urothelial Carcinoma-July 2016 data obtained from TCGA.

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