Tumor microenvironment-based drug discovery: a novel insight into bladder cancer immunotherapy

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To the Editor: Bladder cancer (BCa) is one of the most common urological malignant tumors. With a worldwide incidence of 573,278 cases in 2020, it ranks eleventh among all tumors.¹ Despite having different approved immune checkpoint inhibitors for use among patients with BCa, the response rate to those drugs has remained limited, varying from 20% to 40%.² Therefore, it remains critical to discover new effective immunotherapy drugs to treat BCa. The tumor microenvironment of malignant solid tumors is believed to consist not only of tumor cells but also normal cells, such as stromal cells and immune cells, which have been revealed to play important roles in tumor growth and progression. This indicates the feasibility of designing drugs targeting at those normal cells. Therefore, we propose that it is possible to identify drugs potentially regulative for BCa, based on a newly established algorithm predicting the infiltration level of immune and stromal cells in the tumor microenvironment.³ We anticipate that this algorithm will provide new insight into the immunotherapy of BCa.

Level 3 gene expression data of 393 patients with BCa were identified from The Cancer Genome Atlas program (https://portal.gdc.cancer.gov/) with clinical variables including gender, age, race, histology classification, tumor stage, and survival outcomes. Patients with a survival time period of <1 month were excluded. Immune and stromal scores were calculated to present the level of infiltration of immune and stromal cells in the tumor microenvironment for each patient, using the abovementioned algorithm. The optimal cut-off value was identified as 1895.774 for immune score and −858.8528 for stromal score [Supplementary Figure 1A, http://links.lww.com/CM9/A578]. Kaplan-Meier curves revealed that a higher stromal score (226 patients) was correlated with worse overall survival, but a higher immune score (46 patients) was correlated with improved overall survival [Supplementary Figure 1B, http://links.lww.com/CM9/A578 and 1C, http://links.lww.com/CM9/A578]. Furthermore, the stromal score was associated with tumor stage (P < 0.001), while the immune score was not [Supplementary Figure 1D, http://links.lww.com/CM9/A578 and 1E, http://links.lww.com/CM9/A578].

We identified 136 differential expression genes (DEGs) between groups with high and low stromal scores, and 468 DEGs were found in the immune groups [Supplementary Figure 2A–D, http://links.lww.com/CM9/A578]. Venn plotting eventually identified 562 DEGs after the intersection [Supplementary Figure 2E, http://links.lww.com/CM9/A578]. Kaplan-Meier survival curve screened 123 prognostic genes out of the 562 DEGs (P < 0.050). The survival curve of the Ephrin B2 gene (EFNB2) is shown in Supplementary Figure 2F, http://links.lww.com/CM9/A578.

Protein-protein interaction (PPI) network was constructed (https://string-db.org/) based on 123 prognostic genes to better understand interactions between those genes. We identified the core module with 34 genes of the PPI network [Supplementary Figure 3, http://links.lww.com/CM9/A578]. Enrichment analysis revealed that 34 prognostic genes were correlated with multiple pathways, for instance, hypoxia-induced factor 1 (HIF-1) and tumor necrosis factor signaling pathways [Supplementary Figure 4A and 4B, http://links.lww.com/CM9/A578].

The prognostic value of those 34 core genes was validated in GSE31684, an independent dataset from Gene Expression...
Omnibus (https://www.ncbi.nlm.nih.gov/geo/) involving 78 patients with BCa. After external validation, EFNB2 was the only prognostic gene \((P = 0.036)\) [Supplementary Figure 4C, http://links.lww.com/CM9/A578]. EFNB2 encoded the \(B\) class ephrin, which was previously found to be correlated with \(TP53\) mutation, a well-known tumor suppressor gene, and worse survival of BCa.

Pivot function of the core module was revealed based on ncRNA-mRNA interaction and transcription factor (TF)-mRNA interaction using RNA Interactome Database 2.0 (https://www.rna-society.org/rnainter/) and Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining database v.2 (https://www.grnpedia.org/trrust/). Five ncRNAs and four TFs were associated with the regulation of the core module, and they are visualized in Supplementary Figure 4D, http://links.lww.com/CM9/A578. The five ncRNAs were: (1) metastasis associated lung adenocarcinoma transcript 1, (2) apoptotic BCL2L1-antisense long non-coding RNA, (3) taurine up-regulated gene 1, (4) FOXL1 adjacent non-coding developmental regulatory RNA, and (5) Angelman syndrome chromosome region. The four TFs were: (1) hypoxia inducible factor 1 subunit alpha (HIF1\(\alpha\)), (2) interferon regulatory factor 1, (3) RUNX family transcription factor 3, and (4) signal transducer and activator of transcription 3.

The biological process and disease associated with those ncRNAs and TFs are presented in detail in Supplementary Table 1, http://links.lww.com/CM9/A578. Next, six types of drugs potentially regulative for BCa, including FG-2216, ENMD-1198, Carvedilol, PX-478, and 2-Methoxyestradiol, were located according to the core module and its correlated ncRNAs and TFs using the DrugBank database (https://www.drugbank.ca/). The structure and detailed information about those drugs are displayed in Supplementary Figure 5, http://links.lww.com/CM9/A578 and Supplementary Table 2, http://links.lww.com/CM9/A578.

HIF-1\(\alpha\) is a classic regulator of the transcription of many genes involving tumor angiogenesis, and it is the target of five of the drugs we identified (Carvedilol, FG-2216, ENMD-1198, PX-478, and 2-Methoxyestradiol). Hypoxia is one of the critical characteristics of the tumor microenvironment, leading to malignant progression and development of resistance to anti-cancer therapies together with extracellular acidosis, high lactate levels, etc. In this context, hypoxia-dependent HIF-1\(\alpha\) activation plays a key role in orchestrating a multifaceted (local) suppression of innate and adaptive anti-tumor immune responses. The enhanced HIF-1\(\alpha\) expression activates downstream immune-suppressive effects (recruitment of immune-suppressor cells such as regulatory T cell) and inhibits anti-tumor immune responses (inhibition of immune cells such as CD4\(+\), CD8\(+\) T cells, and antigen-presenting cells). Earlier studies have reported that HIF-1\(\alpha\) was associated with an unfavorable prognosis for patients with BCa. Another study also stated that HIF-1\(\alpha\) could play a critical role in the gemcitabine resistance of BCa. This finding was supported by evidence that the HIF-1\(\alpha\)/multidrug resistance mutation 1 gene pathway conferred chemoresistance to cisplatin in patients with BCa. Therefore, these results enhance the idea that HIF-1\(\alpha\) may be a potential target of immunotherapy for BCa.

Emricasan is a caspase inhibitor and investigational drug for non-alcoholic steatohepatitis. Caspase-8 plays an essential role by regulating the immune response, \(B\) and \(T\) lymphocyte activation, and macrophage differentiation and polarization. Therefore, caspase-8 is an important regulator of immune cell homeostasis and cytokine production, which are the two major components of the tumor microenvironment. This provides the feasibility of Emricasan’s role in improving the immunotherapy efficacy of BCa.

Notably, our drug discovery process is based on transcriptional data, while the targets of the drugs we eventually identified were usually proteins. However, the discordance between the expression of mRNA and protein is not unusual in the research. Certain mechanisms, including translation rates (represented by ribosomes bound to transcripts) and protein stability (involving lysosome and proteasome pathways), have been proposed to explain this discordance but have failed to do so. Recently, Poggio et al. proved that the secretion of protein in the extracellular vesicles could be the cause of the discordance between the expression of mRNA and protein for the same gene.

In conclusion, the current study identified six potentially regulative drugs (FG-2216, Emricasan, ENMD-1198, Carvedilol, PX-478, and 2-Methoxyestradiol) and a potential immunotherapy target (\(EFNB2\)) of BCa based on the concept of the tumor microenvironment. Our findings may provide new insight into the immunotherapy of BCa.

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**Conflicts of interest**

None.

**References**


