An Approach to Study Prognosis of Bacille Calmette-Guérin for Bladder Cancer Treatment

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Abstract

Bladder cancer is a common cancer in the world. One treatment approach, Bacille Calmette-Guérin (BCG), has been used for more than 30 years. There is no prognosis biomarker for BCG treatment. Here we used public data to screen candidate genes as biomarkers for BCG prognosis, presenting the dry-lab way for scientific study which is cost-effective especially for researchers with limited resources. The candidates need further verification on its potential. Sensitive prognosis markers would help more precisive BCG application, which may promote BCG application where it is still limited.

Keywords: Bladder Cancer, BCG, Treatment

Bladder cancer (BC) is a common urologic malignancy in the world [1–3]. BCG is the gold standard option for BC patients classified as intermediate or high risk according to European Association of Urology (EAU) for more than 30 years [4]. It has been verified in multiple studies that BCG is more effective than transurethral resection (TUR) alone or combined with

intravesical chemotherapy [5, 6]. Full BCG includes induction and seven maintenance courses [7]. Clinical trials suggest that BCG immunotherapy reduces BC progression and recurrence, meanwhile extends disease-free survival.

Compared with chemotherapy, BCG provides a different way to kill cancer by stimulating effective anti-cancer immune responses which chemotherapy would not provide.. Studies reveal that there is NMIBC non-responsive to BCG, though there is no reliable biomarkers for predictively patient selection. Besides, It is not unusual that the practices on a disease are quite different in countries for many reasons including technology, economy, culture, etc. BC is such a disease with much differences on diagnosis and treatments between countries. For example, Chinese Bladder Cancer Consortium (CBCC) summarized the clinical features of BC in China [8]. One of the major differences is that Bacille Calmette-Guérin (BCG) which is a common treatment in webstern is barely used for Non-muscle Invasive Bladder Cancer (NMIBC) in China. Therefore, it is necessary to figure out the NMIBC responsive to BCG for more precisive administration, which may help doctors acknowledge BCG better especially in the areas where BCG is not prevalent yet. Here through analysis on public data, we tried to find out whether it is possible to distinguish BCG-responsive NMIBC with certain molecular markers. The study displayed the approach based on public data to screen prognostic markers for BCG treatment.

1 Methods

GSE176178 from GEO (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE176178) includes 40 patients with high grade NMIBC. Whole exome sequencing data on the tumor tissues is accessible in GEO. All patients were treated with induction BCG and grouped by response into durable and non-durable responders.

The study only takes the male data for the well-known different progression of BC in male and female. As to each gene, comparison is performed with Student's t-test between durable and non-durable responders in the statistics software R (Version 4.0.4). With the thresholds of P less than or equal to 0.01 and the absolute value of fold change greater than or equal to 1.5, 55 genes are selected for further biological function analysis. The functional analysis is finished with DAVID (https://david.ncifcrf.gov). The survival data is from bladder cancer in TCGA (https://gdc.cancer.gov).

2 Results

The 55 genes showed quite different expression pattern between BCG responders and BCG non-responders in Figure 1. Most of the genes up-regulated in BCG non-responders, while down-regulated in BCG responders. Three biological pathways were enriched in the functional analysis including ras singaling, positive regulation of MAPK cascade and Cancer Immune.

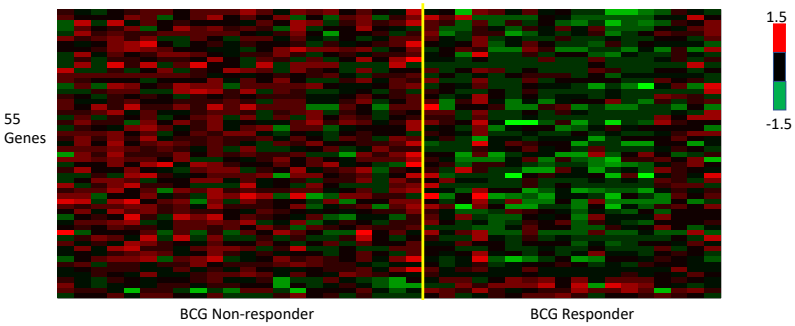
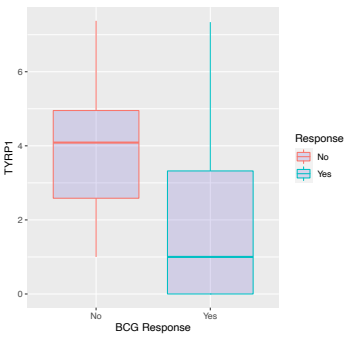
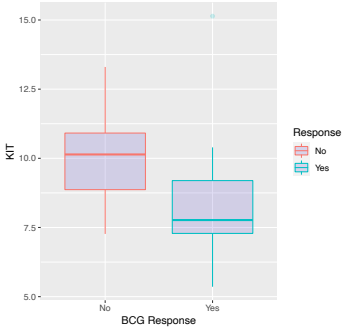


Fig. 1: 55 Genes Expression in Male Samples

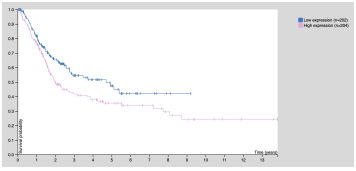
Bar plots were made for TYRP1 and KIT, as shown in Figure 2. The expression bias was shown in the barplots. The survival curves of both genes presented significant differences ($P \leq 0.05$). Survival analysis of TYRP1 took use of 204 high-expression patients versus 202 low-expression patients, of which the cut-off of expression was 0.08 FPKM and P value was 0.019. Survival analysis of KIT took use of 79 high-expression patients versus 28 low-expression patients, of which the cut-off was 0.42 FPKM and P value was 0.031.



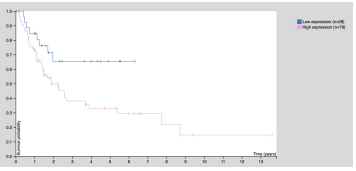
(a) The Comparison of TYRP1 Expression



(b) The Comparison of KIT Expression



(c) The Survival Curve Based on TYRP1



(d) The Survival Curve Based on KIT

Fig. 2: Selected Gene Plots

3 Discussion

BCG has been applied in BC treatment for more than 30 years [7], though there is no prognostic marker yet in practice. A reliable biomarker for BCG would help recognize patients more sensitive to BCG, meanwhile allow people who might be BCG-resistant to turn to other managements in time.

To find possible biomarkers in thousands of genes and proteins, one approach is running high-throughput assays such as whole exome sequencing, whole expression sequencing, whole genome methylation sequencing, etc. Researchers from all over the world have been contributing tons of sequencing and microarray data on many aspects about kinds of diseases, e.g. there are about 4.6 million samples accumalated in GEO. Making full use of those data is a quick manner of test drive without consuming lots of cost and time which is the opportunity especially for researchers in developing areas to contribute their wisdom. That is the right meaning and purpose of contribution and cooperation in scientific community.

In this tiny study, we showed an example to dig in public data. The original authors reported that based on the GEO data, the up-regulation of several inflammatory pathways is the main differences in gene expression between responders and non-responders [9]. JCHAIN, S100A7, CLEC2B and ANXA10 were the chosen expression differences. We found another set of gene changes: TYRP1 and KIT. The survival data from TCGA dataset presented independent evidences on the connection of the both genes to bladder cancer prognosis. The inconsistency results between analyses of same data is not unusual. It suggests researchers to consider carefully in the analysis methods, the stratification in samples and the assay features, etc. The origin of inconsistency here lies in the different sample selection. Only males in the data was chosen by us since it is well known that BC behaves quite different between genders, so we reasoned that the progression of BC in male and female may be not same and single gender data might be less noise for prognostic marker screen, which is supported by no significant survival analysis on gene expression in original data. In conclusion, it is not pure duplicate work to dig public data. On the contrary, viewpoints of discrepancy may disclose novel facts.

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