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Keywords: the cancer genome atlas; genomics; proteomics; methylation; clinical significance

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Title: Exploring cancer genomic data from The Cancer Genome Atlas Project.

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Running Title: Analysis of TCGA data

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ABSTRACT

The Cancer Genome Atlas (**TCGA**) has compiled genomic, epigenomic, and proteomic data from more than 10,000 samples derived from 33 types of cancer, aiming to improve our understanding of the molecular basis of cancer development. Availability of these genome-wide information provides an unprecedented opportunity for uncovering new key regulators of signaling pathways or new roles of pre-existing members in pathways. To take advantage of the advancement, it will be necessary to learn systematic approaches that can help to uncover potential biomarkers reflecting genetic alterations, prognosis, or response to treatments. This minireview describes the updated status of TCGA project and explains how to use TCGA data.

INTRODUCTION

Genome era began with the successful completion of the Human Genome Project with the physical and genetic mapping of genes (1-3), followed by further development of technologies for gene expression profiling, genome-wide copy number alteration analysis, and 2nd generation of sequencing enabled the comprehensive characterization of entire genomes. The blueprint of the human genome has expedited our efforts to understand the molecular pathways involved in human disease. This information promises to improve our understanding of the genetic and epigenetic alterations accountable for many human diseases, as well as ultimately provide biological functions of all genetic elements. Two major branches of genomics include 1) structural genomics that catalogues all genetic elements by mapping and sequencing, 2) functional genomics that try to uncover the roles of genes in biological systems. In addition to DNA sequencing, one of the most important technologies for genomics is microarrays, which can measure the expression of entire genes, copy number alteration and methylation in entire genome simultaneously. Because gene expression patterns well reflect the functional activity of genes, gene expression profiling has been extensively used for screening phenotype-associated genes and uncovering functions of newly discovered genes or new functions of known genes.

In recent years, genome-wide analysis and the use of publicly available high-throughput data facilitated the identification of genes having unexpected roles in the cellular and physiological process or association with malignant diseases or with therapeutic targets of diseases (4-6). Thus, in-depth analysis of multiple genomic data will undoubtedly reveal novel insights into the regulation of many signaling pathways or novel key regulators of the pathways.

In this review, I will provide a short description of major progress on cancer genomics, particularly in The Cancer Genome Atlas (TCGA) project. Furthermore, I will provide description on the data generated by different platforms and analytical tools that have been developed through the progression of TCGA projects.

The Cancer Genome Atlas (TCGA)

The Cancer Genome Atlas (TCGA) Project is a landmark research program supported by the National Human Genome Research Institute and National Cancer Institute at the National Institutes of Health. TCGA was launched to facilitate the comprehensive understanding of the cancer genetics using state-of-art genomic technologies and analysis tools to catalogue all of the potential cancer drivers, identify robust prognostic and predictive biomarkers and novel druggable therapeutic targets, and uncover molecular subtypes of tumors that are different in prognosis and response to treatments. By using various different platforms, TCGA currently gathers many different genome-wide data including mRNA expression, microRNA expression, somatic mutations, copy-number alteration, and promoter methylation. In addition, it also generates proteomic data by using reverse phase protein arrays (RPPA) technology. The project plans to collect genomic and proteomic data from more than 500 tissues per cancer type and release the data to public without any restriction in use of the data. In 2005, a pilot study (phase I) started aiming to test the feasibility of ideas and develop the research infrastructure by characterizing few selected cancer types that are understudied: glioblastoma, lung squamous cell cancer, and ovarian cancers (7-10). Phase 2 study was started in 2009 and expanded to additional cancer types (33 cancer types).

This ambitious project has identified novel driver genes and biomarkers on the basis of genomic, transcriptomic, proteomic and epigenomic alterations. Some of findings are clinically relevant and unexpected. For example, we now learned that non-hypermutated adenocarcinomas of the colon and rectum are not distinguishable at the genomic level (11). In lung squamous cell cancer, while *KRAS* and *EGFR* mutations, most commonly activated

oncogenes in lung adenocarcinoma, are extremely rare, alterations in the FGFR kinase family are common (10). Thus, massive data from a large number of tissues created an unprecedented opportunity for taking an integrated approach toward a systems-level understanding of disruptions in cellular and molecular pathways in cancer.

TCGA has established a pipeline for collecting and processing tissues from numerous source sites (tissue banks at hospitals), generation of high quality genomic and proteomic data, and distribution and analysis of the data. Most importantly, major bodies for data generation and analysis are consisted of the Genome Characterization Centers (GCCs), Genome Sequencing Centers (GSCs) and Genome Data Analysis Centers (GDACs). The GCCs aim to identify all genomic alterations in the tumors in each cancer type. Each GCC uses most advanced platform technologies to generate mRNA and miRNA expression data, DNA methylation data, and copy number alteration data. The genetic changes identified by the GCCs are further characterized by the GSCs that perform large-scale genomic sequencing using the latest sequencing technologies to identify small genomic changes that could play a role in cancer. All of the data generated by the GCCs and GSCs on the multiple genomic platform technologies from thousands of tissue samples are transferred to GDAC through Data Coordinating Center (DCC). The GDACs are responsible for analysis of the data and development of new bioinformatics tools that can facilitate use of TCGA data by the entire research community.

Types of data generated from TCGA project

Six different platform data are currently generated from GCC and GSC and available to general public. These include somatic mutation data, mRNA and miRNA expression data, DNA methylation data, copy number alteration data, and proteomic data.

Whole exome sequencing data: Majority of mutation data were generated by whole exome sequencing using second-generation DNA sequencing instruments (mostly Illumina and ABI SOLiD). Whole exome sequencing analysis is carried out by sequencing the DNA coding for protein products, but not DNA sequences that do not directly code for proteins. However, about 10% of samples in TCGA project underwent whole genome sequencing, which sequences every base-pair of DNA and that can reveal any alteration in regulatory regions of genome.

mRNA expression data: mRNA expression profile data were first generated by using microarray technologies from Affymetrix or Agilent, but RNA sequencing (RNA-seq) technology from Illumina was used in later stage of TCGA project. RNA-seq technology has several advantages over microarray platform as it can quantify rare and common transcripts, alternative splicing, previously unrecognized transcripts, gene fusions, as well as non-coding RNAs. It can also quantify distribution of somatic mutations and edited RNAs (12).

microRNA expression data: microRNA (miRNA) is a small non-coding RNA (~22 nucleotides in size) that regulates other genes through post-transcriptional manner (13). miRNA expression profile data were generated by directly sequencing small molecule RNAs using

RNA-seq technology from Illumina. These data were separately processed and maintained from data from mRNA-seq data as their biological and molecular characteristics are different from coding RNAs.

DNA methylation data: DNA methylation is an epigenetic mark which is frequently associated with transcriptional activity of genes. TCGA DNA methylation data were initially generated by using Illumina 27K DNA methylation array (HumanMethylation27 containing 27,578 probes in 14,495 genes). Later, it was replaced by 450K methylation arrays (HumanMethylation450 containing 485,512 probes covering 99% RefSeq genes).

DNA copy number alteration data: Copy number alteration is probably most frequent genetic events during the course of tumor development. Copy number data were generated by using Affymetrix SNP 6.0 arrays containing 1.8 million genetic markers, including more than 906,600 single nucleotide polymorphisms (SNPs) and more than 946,000 probes for the detection of copy number variation.

Reverse-phase protein array (RPPA) data: RPPA is an antibody-based quantitative methods assessing hundreds of protein markers in thousands samples in a cost-effective, sensitive and high-throughput manner (14). This technology has been extensively validated for both cell line and patient samples, and its applications range from building reproducible prognostic models to assessing underlying biology associated with prognosis. Current RPPA data from TCGA project include expression and modification of ~200 proteins.

In addition to genomic and proteomic data, TCGA data also include slide images for histopathology and details on patients information such as tumor stages, races, potential etiology, treatments and survival.

Where to get TCGA data

All of genomic, proteomic, and clinical data from TCGA project were available from TCGA data portal site. However, as of July 15th, 2016, the TCGA Data Portal is no longer operational and all TCGA data now resides at the Genomic Data Commons (GDC, https://gdc-portal.nci.nih.gov/). While a vast majority of TCGA data in the GDC are publically available without restriction, meaning that no authentication or authorization is necessary to access it, some of the data are controlled access, meaning that special authorization process is necessary to access the data. Access to controlled data is typically granted by program-specific Data Access Committees (https://gdc.nci.nih.gov/access-data/obtaining-access-controlled-data). Public availability of the data is ruled by the NIH Genomic Data Sharing Policy (https://gds.nih.gov/). Open access data typically includes the data that cannot identify individuals such as high level genomic and proteomic data as well as most clinical and all biospecimen data elements. Controlled data includes individually identifiable data such as low level genomic sequencing data, germline variants, SNP6 genotype data, and certain clinical data elements.

Processed high level data are also available from UCSC Cancer Genomics Browser (https://genome-cancer.ucsc.edu/). It offers more user-friendly processed data and limited visualization tools are also available. Histology information is also available from The Cancer Digital Slide Archive, CDSA (http://cancer.digitalslidearchive.net/), which provides the

interactive tools for viewing and annotating diagnostic and tissue slide images from TCGA project (15). In addition to genomic, proteomic, and clinical data, TCGA also offers radiological imaging data from TCGA patients through The Cancer Imaging Archive, TCIA (http://www.cancerimagingarchive.net) in order to stimulate imaging phenotype-genotype study (16).

How to analyze TCGA data

Comprehensive genomic data from large number of patients would undoubtedly improve our knowledge in understanding of cancer-related genes and their clinical relevance. However, analysis of such "big data" would require substantial skills in computational tools, statistics, and programming languages. Thus, it would be necessary to develop easy-to-use and intuitive genomic tools that can help researchers or clinicians in analysis and interpretation of all the data types in a meaningful way. TCGA provides intuitive web-based tools.

The cBioPortal for Cancer Genomics (http://cbioportal.org) offers probably best web-based tool for beginners who have limited experience in analysis of genomic data and only wish to analyze limited number of genes (17). The cBioPortal is an open-access resource developed by investigators at the Memorial Sloan-Kettering Cancer Centre (MSKCC). It allows users to search gene(s) of interest in certain cancers or all cancers in TCGA data and provides a flexible interface to multiple data sets and easy-to-use visualization options. The cBioportal offers unique analysis and visualization tools such as MEMo (Mutual Exclusivity Modules)

analysis, correlation plots for expression and copy number alteration or methylation of genes, assessing clinical relevance of genes by Kaplan-Meier plots, co-expression analysis, network analysis. In addition, it also offers highly useful OncoPrint diagrams that are an intuitive diagram of genomic alterations such as somatic mutations and copy number alterations across a set of samples. Mutationmapper provides summary diagram of all mutations on a linear protein map and links to protein 3D structure database to examine potential effects of mutations. More importantly, all analyzed data can be downloaded in table format for further analysis.

The Broad GDAC Firehose (http://gdac.broadinstitute.org/) is a web portal site that has been developed by the Broad Institute, aiming to deliver automated analyses of the TCGA data to general users. It provided preprocessed annotated data and association analysis across all types of data including clinical data. For example, it can provide list of genes whose copy number alteration, methylation, mRNA expression, and mutations are significantly correlated with tumor stages, survival of patients, sex, ages, or ethnic groups. Expression of genes of interest across all cancer types can be also easily assessed in firebrowse (http://firebrowse.org/).

PROGgeneV2 (http://watson.compbio.iupui.edu/chirayu/proggene/database/) provide survival analysis of patients from multiple cohorts in database (18). Users can choose either single gene or set of genes to estimate their association with prognosis of patients. Because

typical molecular biologists would not have good background on statistics that is necessary to run survival analysis, it would be useful tool for them.

Mexpress (http://mexpress.be/) provides easy-to-use data visualization tool of the TCGA data including mRNA expression, DNA methylation, and clinical data (19). In addition, it also provide the correlation among data sets.

The Cancer Proteome Atlas (TCPA)

(http://app1.bioinformatics.mdanderson.org/tcpa/_design/basic/index.html) is data portal for proteomic data from TCGA project (20). It provides correlation analysis between proteins and association of proteins with prognosis of patients. In addition to TCGA data, it also provide data from established cancer cell lines.

Exploration of genomic data

Analysis tools from TCGA project developed to make that basic scientists without training in informatics, statistics, and clinical knowledge can analyze the data and interpret the results. The potential involvement of genes of interest in cancer development can be easily assessed. For example, genetic alterations of peroxiredoxin family in all cancer types can be assessed through cBioPortal (**Figure 1A**) and alterations of individual genes in certain cancer type (i.e., ovarian cancer) are visualized in oncoprint format (**Figure 1B**). Furthermore, the clinical relevance of alteration is estimated and displayed in Kaplan-Meier plots (**Figure 1C**). Clinical association of genes of interest can be further validated by using tools in

PROGgeneV2. Correlation between different genomic data is also readily visualized through cBioProtal and Firehose (**Figure 2**).

CLOSING REMARK

TCGA is an unprecedented powerful public resource of cancer genomic data providing researchers with a great opportunity to increase present knowledge on cancer. Multi-layer analyses performed on different platforms reflecting distinct biological characteristics provide a better understanding of cancer biology, leading to improvement in patient stratification, identification of novel prognostic or predictive markers, and finding novel potentially druggable therapeutic targets. The translation of genomic knowledge into biological insights will move these new findings to the next level and guide to a new era in data-driven molecular biology.

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FIGURE LEGENDS

Figure 1. Visualization of analyzed data. (A) The spectrum of genetic alteration in PRDX genes in different cancer types. (B) Genetic alterations of PRDX genes in ovarian cancer. (C) Kaplan-Meier plot of patients with ovarian cancer stratified according to genetic alteration of PRDX genes.

Figure 2. Scatter plots between mRNA expression and copy number alteration of PRDX1 and PRDX2 in ovarian cancer.

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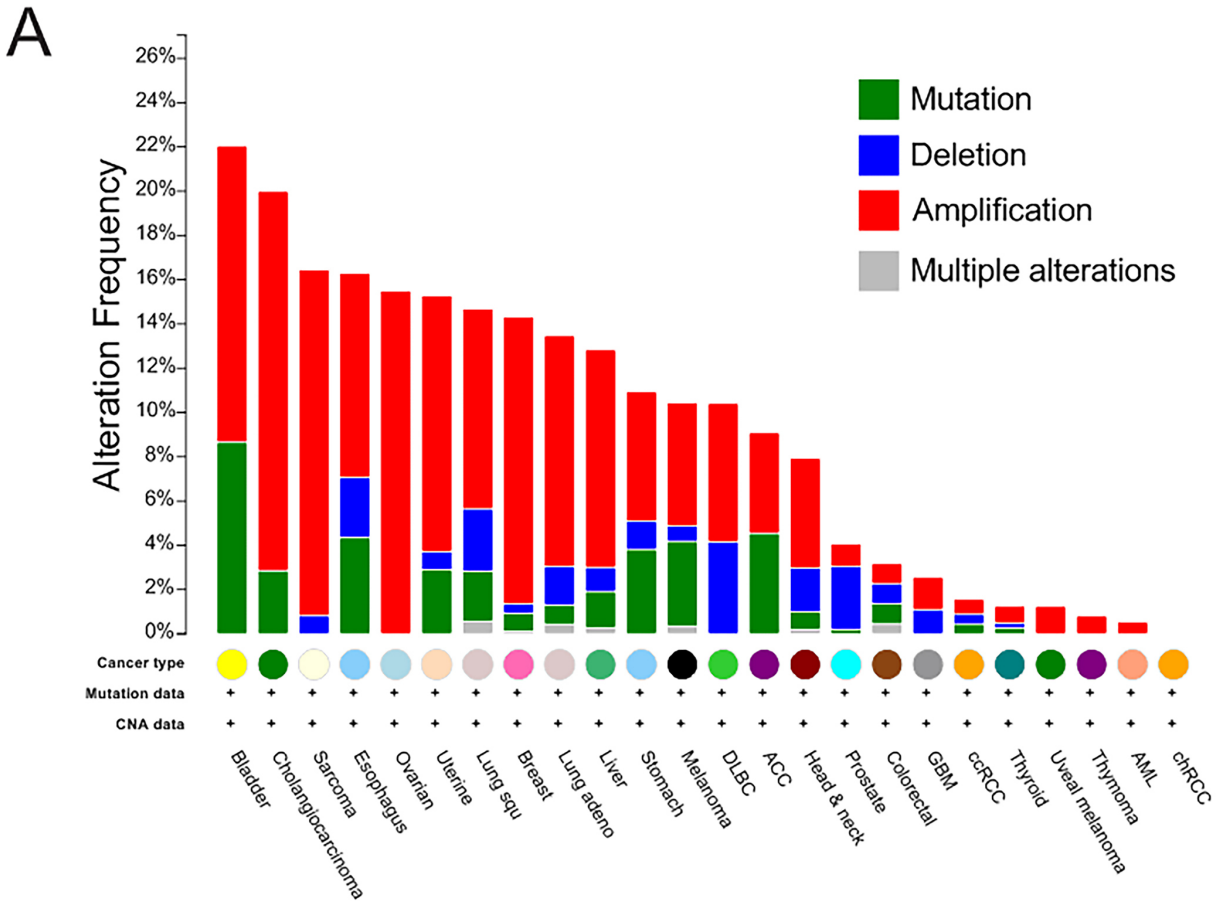
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CNA data
Bladder
Cholangiocarcinoma
Sarcoma
Esophagus
Ovarian
Uterine
Lung squ
Breast
Lung adeno
Liver
Stomach
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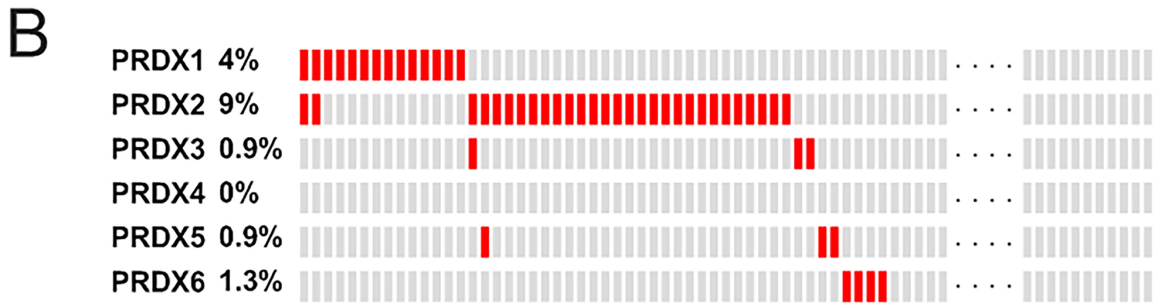
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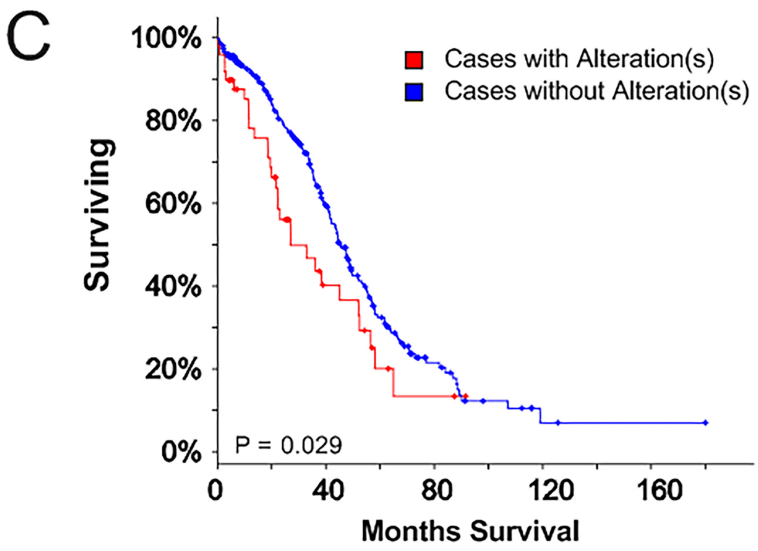
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Fig. 1

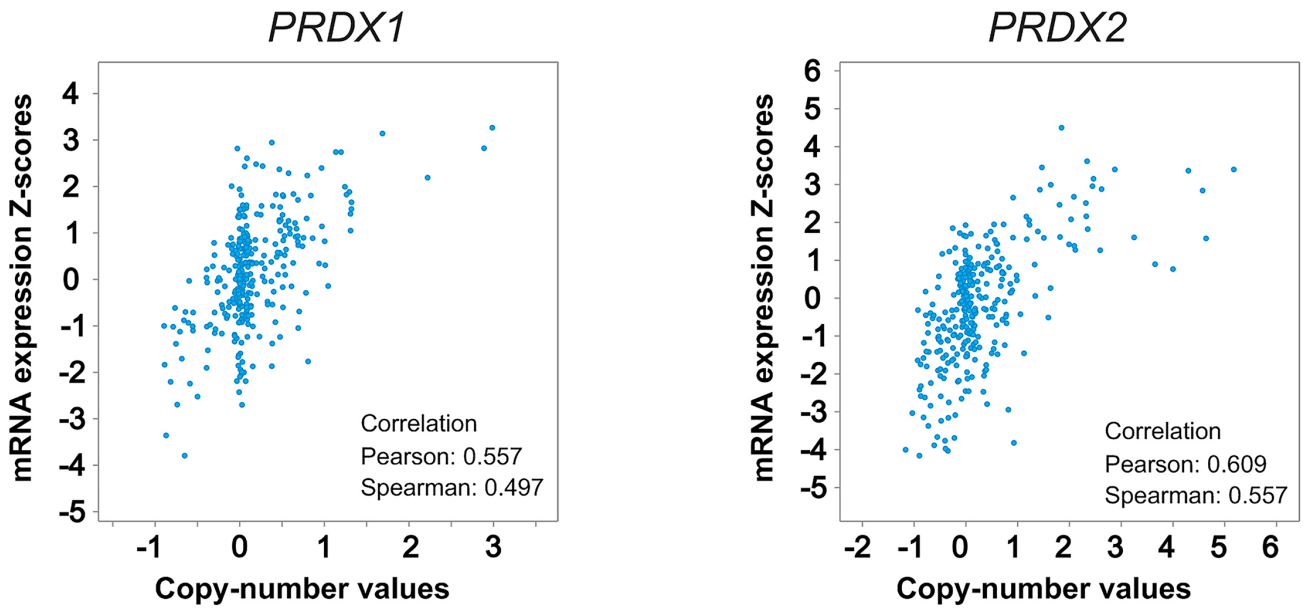
PRDX1
mRNA expression Z-scores
Copy-number values
Correlation
Pearson: 0.557
Spearman: 0.497
PRDX2
mRNA expression Z-scores
Copy-number values
Correlation
Pearson: 0.609
Spearman: 0.557

Fig. 2