The SMART App: an interactive web application for comprehensive DNA methylation analysis and visualization

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Abstract

Background: Data mining of The Cancer Genome Atlas (TCGA) data has significantly facilitated cancer genome research and provided unprecedented opportunities for cancer researchers. However, existing web applications for DNA methylation analysis do not adequately address the need of experimental biologists, and many additional functions are often required.

Results: To facilitate DNA methylation analysis, we present the SMART (Shiny Methylation Analysis Resource Tool) App, a user-friendly and easy-to-use web application for comprehensively analyzing the DNA methylation data of TCGA project. The SMART App integrates multi-omics and clinical data with DNA methylation and provides key interactive and customized functions including CpG visualization, pan-cancer methylation profile, differential methylation analysis, correlation analysis and survival analysis for users to analyze the DNA methylation in diverse cancer types in a multi-dimensional manner.

Conclusion: The SMART App serves as a new approach for users, especially wet-bench scientists with no programming background, to analyze the scientific big data and facilitate data mining. The SMART App is available at http://www.bioinfo-zs.com/smartapp.

Keywords: TCGA, DNA methylation, Gene expression, Web application

Introduction

All cancers arise as a result of the accumulation of somatic mutations, copy number alterations, and epigenetic modifications that alter transcription and protein expression. Thus, studies of molecular features such as DNA methylation may reveal the underlying mechanisms of carcinogenesis and progression. DNA methylation, the addition of a methyl group to DNA, plays a critical role in regulating gene expression [1]. It has been reported that DNA methylation at the promoter regions is often negatively correlated with gene expression while DNA methylation in gene bodies is often positively correlated with gene expression [2]. Abnormal DNA methylation patterns are found in every type of human cancer [3]. Many previous studies have shown that DNA methylation is involved in many aspects of carcinogenesis and provides potential biomarkers for evaluating the diagnosis and prognosis of cancer [4–6]. A recent study has also shown the association between DNA methylation and somatic copy number aberration, suggesting a much more complex mechanism beyond this modification [7].

The Cancer Genome Atlas (TCGA), a project supported by the National Cancer Institute (NCI) and National Human Genome Research Institute (NHGRI), hosts tremendous amount of multi-omics data and allows systematic study of the genetic or epigenetic basis of cancer [8]. However, accessing and analyzing the DNA methylation data from TCGA database is quite difficult for those scientists who have no computational background. Therefore, constructing easy-to-use applications for analyzing the DNA methylation data of TCGA database is demanded.
and shores is displayed below (Fig. 1). This segment plot with transcripts, a detailed segment plot highlighting the genomic locations along the chromosomes may reflect the genomic mechanisms leading to specific methylation characteristics [14]. Therefore, the SMART App allows users to set custom cut-off values for a given cancer type to dynamically obtain differentially methylated CpGs and their chromosomal distributions (Fig. 2). The delta |Beta-value|/delta |M value| of each probe is calculated as the mean Beta-value/M value in tumor samples minus the mean Beta-value/M value in normal samples. *p* value is calculated using the Wilcoxon rank sum test, and is adjusted using the Benjamini–Hochberg method. Moreover, for users who only want to visualize specific CpGs, the SMART App offers an extra function that allows users to draw CpG flexibly. The detailed description can be found at the website.

**Methylation DIY**

This module provides functions for users to comprehensively analyze DNA methylation taking other omics data and clinical stages into consideration. The first panel generates custom box plots for users for compare CpGs of genes between normal and tumor samples in a given cancer type. Users can select multiple probes at

| Differential CpGs |

Differential analysis is a common approach in cancer research by comparing tumor samples vs. normal samples for identifying aberrantly methylated CpGs. Meanwhile, clustering of the CpGs with similar methylation patterns along the chromosomes may reflect the genomic mechanisms leading to specific methylation characteristics [14]. Therefore, the SMART App allows users to set custom cut-off values for a given cancer type to dynamically obtain differentially methylated CpGs and their chromosomal distributions (Fig. 2). The delta |Beta-value|/delta |M value| of each probe is calculated as the mean Beta-value/M value in tumor samples minus the mean Beta-value/M value in normal samples. *p* value is calculated using the Wilcoxon rank sum test, and is adjusted using the Benjamini–Hochberg method. Moreover, for users who only want to visualize specific CpGs, the SMART App offers an extra function that allows users to draw CpG flexibly. The detailed description can be found at the website.

| Methylation DIY |

This module provides functions for users to comprehensively analyze DNA methylation taking other omics data and clinical stages into consideration. The first panel generates custom box plots for users for compare CpGs of genes between normal and tumor samples in a given cancer type. Users can select multiple probes at
the same time for easy visibility and interpretation. The returned box plots will display all the selected probes plus an aggregation box plot showing the mean/median methylation of all the selected probes. The second panel plots methylation by pathological stages based on the TCGA clinical data. Two options are available, namely, major stage and sub-stage. For example, if users choose major stage for plotting, stage IIA/IIB will be included in the stage II group. Here, using SMART App, we can easily find that TRIM58 (cg10983544) is much hyper-methylated in stage II group in lung squamous cell carcinoma, indicating its clinical relevance (Fig. 3b, \( p \) value = 0.016). Somatic mutations can also affect DNA methylation. To help users study the effect of somatic mutations on DNA methylation, the SMART App offers a function for plotting box plots comparing methylation between mutation and wild-type groups. For example, IDH1 mutation can cause hyper-methylation in lower grade glioma (LGG) [15]. When IDH1 is selected, the returned box plots showed that cg07640666, cg17353896 and cg24324379 were significantly hyper-methylated in the mutation group (Fig. 3a, \( p \) value < 0.05). Sun et al. observed the correlation between CNV and methylation and discussed the possible mechanisms relating to this event [7]. Here, the SMART App provides a function for researchers to study the possible association between CNV and DNA methylation. The results are displayed as box plots...
plots showing the correlation between CNV and methylation. With the SMART App, it is very interesting to observe that TRIM58 (cg04902327) shows a lower level of methylation with low-level copy number amplification, whereas other CpGs of TRIM58 show a positive correlation with CNV in lung squamous cell carcinoma (Fig. 3c, p value < 0.05).

Correlation DNA methylation is often correlated with gene expression. The correlation function of SMART App performs correlation analysis between gene expression and methylation for any given sets of TCGA, using methods including Pearson, Spearman, and Kendall correlation statistics. The UCSC Xena provides the re-computed expression data of TCGA for 198,619 transcripts. Accordingly, there are two levels available, and one can choose to analyze the correlation at gene level or transcript level. When analyzing the correlation at transcript level, a segment plot highlighting the genomic locations of the transcript and CpGs will be displayed, and the distances of each probe to TSS will also be shown in the table below for users to locate the ones at the promoter region. The results are displayed as scatter and distribution plots (Fig. 4 and Additional file 1: Figure S1).

Survival The SMART App performs overall survival (OS) and disease-free internal (DFI)-related survival analysis based on methylation levels. This function allows users to select their custom cancer types for overall or disease-free survival analysis. Cox regression analysis is a popular method for evaluating the prognostic value of individual variables. To efficiently analyze the survival significance of methylation, the SMART App offers both univariate and multivariate Cox regression analyses. When performing multivariate Cox regression analysis, users can adjust for potential confounding factors, including age, gender, race and pathological stage. Users can copy and paste a list of CpGs into the box, and select the cancer type of interest to conduct Cox regression analysis. The hazard ratio, 95% confidence interval, z score, and p value will be given. Once users have identified the significant variables, they can use the SMART App to draw survival curves. The thresholds for high/low methylation level cohorts can be adjusted by users.

Comparison with existing tools Web tools to analyze DNA methylation of TCGA project include methHC, Wanderer, MEXPRESS, and MethSurv. MethHC was introduced in 2014 and enables users to identify highest/lowest methylated genes, perform hierarchical cluster analysis, explore methylation profile across tumors and conduct correlation analysis. However, the latest update of methHC was in 2014. Wanderer is an interactive web application to explore DNA methylation and gene expression. It provides a single-page interface to explore DNA methylation in a regional framework. MEXPRESS is a data visualization tool for DNA methylation analysis and was first introduced in 2015. Now, it has been updated, adding more data and generating fancier figures. MethSurv is a shiny application that mainly focuses on the clinical impacts of DNA methylation. While these tools are extraordinarily valuable, many extra functions are not adequately addressed by them. M value has been reported to be more statistically valid for the differential analysis [16]. Although differential analyses are commonly performed by these tools, none of them allow users to use the M value for differential analysis. None of these tools allow users to pick a cancer type and visualize the chromosomal distribution of the aberrantly methylated CpGs. In addition, none of the existing tools allow users to analyze the correlation between methylation and expression at transcript level. Besides, none of the tools provide customizable selection of methylation thresholds for patient cohort partitioning in survival curves plotting. A detailed comparison is shown in Table 1.

Discussion The SMART App is an interactive web application for DNA methylation analysis based on the TCGA database. The SMART App enables experimental biologists without any computational programming background to perform various analyses relating to DNA methylation in diverse cancer types. Using the SMART App, one can easily explore the large DNA methylation data, ask specific scientific questions, and validate their findings. For example, one can easily find that CpGs such as
cg10983544 and cg20429172 are located at the promoter region of the transcript of TRIM58, and may ask whether these CpGs are aberrantly methylated and whether the methylation changes of these CpGs will lead to gene expression alterations. One can also identify significantly hyper- and hypo-methylated CpG-based custom thresholds. Moreover, one can explore the correlation between methylation and other omics and clinical data, analyze the prognostic value of CpGs and draw survival curves. Meanwhile, the flexible customization parameters of the SMART App also enable users to customize the result visualization. The SMART App is a user-friendly and intuitive tool for unlocking the potential value of the genomic data in TCGA. It complements well with other available tools.

**Conclusion**

The SMART App is a web-based tool to explore and interpret the DNA methylation data across 33 cancer types from TCGA database. The source code of the
SMART App is available for users to download under GPLv3 license.

Methods

The SMART App is developed entirely in the R programming language using the Shiny framework and is freely available for all users. There is no login requirement for accessing any features in the SMART App. The SMART App has been most extensively tested in a Safari browser environment and is also compatible with other popular web browsers such as Chrome, Firefox, and Internet Explorer.

The data used in the SMART App are directly pulled down from the TCGA Pan-Cancer cohort of UCSC Xena public data hubs (https://xenabrowser.net) upon users’ request using UCSCXenaTools, including gene expression (TOIL re-computed TPM), transcript expression (TOIL re-computed TPM) [17], DNA methylation (Human-Methylation450k; Primary Solid Tumor and Solid Normal Tissue), somatic mutation (Gene-level non-silent mutation), copy number variation (Gene-level GISTIC2 thresholded), phenotype and clinical information [18]. Gene-level non-silent mutation and gene-level thresholded copy number variation are used because they offer easy-to-interpret values (i.e., 0 for wild type and 1 for mutation for somatic mutation; homozygous deletion (−2), single copy deletion (−1), diploid normal copy (0), low-level copy number amplification (+1) and high-level copy number amplification (+2) for copy number variation). For methylation probes, we used the hg38 coordinates provided by Zhou et al. (http://zwdzd.github.io/InfiniumAnnotation) [19]. Gene, transcript and exon coordinates were obtained from GENCODE (https://www.gencodegenes.org, Release 31, GRCh38.p12).

Both Beta-value and M value are commonly used in DNA methylation analysis. The M value has been reported to have a more dynamic range, and is more appropriate for statistical analysis [16]. Whereas the Beta-value is much more biologically interpretable. Therefore, these two types of methylation values are available in the SMART App.

The SMART App outputs consist of figures and tables, which are available for users to download. Figures are rendered as Portable Document Format (PDF), which can be further edited using Adobe Illustrator. Tables are generated by DT R package (https://rstudio.github.io/DT/) allowing for data querying and selection.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13072-019-0316-3.

Table 1 Functionalities comparison between the SMART App and other tools

|                        | SMART App | methHC | Wanderer | MEXPRESS | MethSurv |
|------------------------|-----------|--------|----------|----------|----------|
| Data source            | TCGA      | TCGA   | TCGA     | TCGA     | TCGA     |
| Latest update          | 2019      | 2014   | 2018     | 2019     | 2018     |
| Genomic location visualization | Yes    | No     | Yes      | Yes      | No       |
| Pan-cancer methylation profile | Yes   | Yes    | No       | No       | No       |
| Differential analysis  | Yes       | Yes    | Yes      | Yes      | Yes      |
| Correlation with other omics data | Yes   | No     | No       | Yes      | No       |
| Correlation with gene expression | Yes   | Yes    | Yes      | Yes      | Yes      |
| Correlation with transcript expression | Yes | No     | No       | No       | No       |
| Cox regression analysis| Yes       | No     | No       | No       | Yes      |
| Survival analysis with custom threshold | Yes | No     | No       | No       | Yes      |
| Hierarchical cluster analysis | No    | Yes    | No       | No       | Yes      |
| Methylation M value    | Yes       | No     | No       | No       | No       |

A yes means this function is available.

Additional file 1: Figure S1. Distribution plots showing the correlation between expression and Methylation. Each bar represents a sample, the names of the gene/transcript and Cpgs are shown on the right, the methylation and expression values are shown on the left. The samples are reordered according to the expression value. A. Gene-level distribution plot. B. Transcript-level distribution plot.

Abbreviations

TCGA: The Cancer Genome Atlas; SMART: Shiny Methylation Analysis Resource Tool; CNV: copy number variation.

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Authors’ contributions

Conceived and designed the study: YL, DG and CL. Built the website: YL. Wrote the paper: YL, DG and CL. All authors read and approved the final manuscript.
Availability of data and materials
Not applicable.

Ethics approval and consent to participate
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Consent to publish
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Competing interests
The authors declare that they have no competing interests.

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