Materials Design Analysis Reporting (MDAR) Checklist for Authors

The MDAR framework establishes a minimum set of requirements in transparent reporting applicable to studies in the life sciences (see Statement of Task: doi:10.31222/osf.io/9sm4x.). The MDAR checklist is a tool for authors, editors and others seeking to adopt the MDAR framework for transparent reporting in manuscripts and other outputs. Please refer to the MDAR Elaboration Document for additional context for the MDAR framework.

Materials

Antibodies	Yes (indicate where provided: section/paragraph)	n/a
For commercial reagents, provide supplier name, catalogue number and RRID, if available.		N/A

Cell materials	Yes (indicate where provided: section/paragraph)	n/a
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID		N/A
Primary cultures: Provide species, strain, sex of origin, genetic modification status.		N/A

Experimental animals	Yes (indicate where provided: section/paragraph)	n/a
Laboratory animals: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID		N/A
Animal observed in or captured from the field: Provide species, sex and age where possible		N/A
Model organisms: Provide Accession number in repository (where relevant) **OR** RRID		N/A

Plants and microbes	Yes (indicate where provided: section/paragraph)	n/a
Plants: provide species and strain, unique accession number if available, and source (including location for collected wild specimens)		N/A
Microbes: provide species and strain, unique accession number if available, and source		N/A

Human research participants	Yes (indicate where provided: section/paragraph)	n/a
Identify authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	The study was approved by the clinical research ethics committee of the first affiliated hospital, college of medicine, Zhejiang University. (Please refer to Method/Patient information and study design, 2nd paragraph)	
Provide statement confirming informed consent obtained from study participants.	All patients provided written informed consent, in accordance with the Declaration of Helsinki. (Please refer to Method/Patient information and study design, 2nd paragraph)	
Report on age and sex for all study participants.	In total 49 participants with a mean age of 63 years old (range, 34-80) and 47 of them are males (96%). (Please refer to Result/ Demographic and clinicopathological characteristics of patients and Table 1)	

Design

Study protocol	Yes (indicate where provided: section/paragraph)	n/a
For clinical trials, provide the trial registration number **OR** cite DOI in manuscript.		N/A

Laboratory protocol	Yes (indicate where provided: section/paragraph)	n/a
Provide DOI or other citation details if detailed step-by-step protocols are available.		N/A

Experimental study design (statistics details)	Yes (indicate where provided: section/paragraph)	n/a
State whether and how the following have been done**, or** if they were not carried out.		
Sample size determination	49 LUSC patients (Please refer to Method/Patient information and study design, 2nd paragraph)	
Randomisation		N/A
Blinding		N/A
Inclusion/exclusion criteria	Inclusion： resectable LUSC (Please refer to Method/Patient information and study design, 2nd paragraph)	

Sample definition and in-laboratory replication	Yes (indicate where provided: section/paragraph)	n/a
State number of times the experiment was replicated in laboratory		N/A
Define whether data describe technical or biological replicates		N/A

Ethics	Yes (indicate where provided: section/paragraph)	n/a
Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	The study was approved by the institutional review board of Zhejiang University. Reference no :The first Affiliated Hospital of Zhejiang University clinical research ethics 2003 No.0598	
Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.		N/A
Studies involving specimen and field samples: State if relevant permits obtained, provide details of authority approving study; if none were required, explain why.	The study was approved by the clinical research ethics committee of the first affiliated hospital, college of medicine, Zhejiang University. All patients provided written informed consent, in accordance with the Declaration of Helsinki. (Please refer to Method/Patient information and study design, 2nd paragraph)	

Dual Use Research of Concern (DURC)	Yes (indicate where provided: section/paragraph)	n/a
If study is subject to dual use research of concern, state the authority granting approval and reference number for the regulatory approval		N/A

Analysis

Attrition	Yes (indicate where provided: section/paragraph)	n/a
State if sample or data point from the analysis is excluded, and whether the criteria for exclusion were determined and specified in advance.		N/A

Statistics	Yes (indicate where provided: section/paragraph)	n/a
Describe statistical tests used and justify choice of tests.	Principal component analysis (PCA) was applied for subgrouping samples. The "limma" package (version: 3.460) in R software was used to study the differentially methylation. Differences were calculated by Fisher's exact test for proportions of the variables across groups. For two continuous variables, pearson's correlation analysis was applied. For DNA methylation levels between two groups and three groups, student's t-test and multiple paired were applied, respectively. For continuous variables between two groups and across three groups, the wilcoxon rank-sum test and ANOVA was performed, respectively. Analyses were performed in R version 3.3.3 software, with two-sided P values less than 0.05 considered statistically significant. (Please refer to Method/ Statistical analysis)	

Data Availability	Yes (indicate where provided: section/paragraph)	n/a
State whether newly created datasets are available, including protocols for access or restriction on access.	The data underlying this article will be shared on reasonable request to the corresponding author. (Please refer to Data availability statement)	
If data are publicly available, provide accession number in repository or DOI or URL.		N/A
If publicly available data are reused, provide accession number in repository or DOI or URL, where possible.		N/A

Code Availability	Yes (indicate where provided: section/paragraph)	n/a
For all newly generated code and software essential for replicating the main findings of the study:		
State whether the code or software is available.	Raw data was trimmed by Trimmomatic (v.0.32), and then aligned by BWA-meth (v.0.2.2). After alignment, PCR duplicates were marked with Samblaster (v.0.1.20). The low mapping quality (MAPQ <20) or improper pairing reads were cleared by Sambamba (v.0.4.7) from the further analyses. (Please refer to Bisulfite targeted sequencing and methylation data processing, 2nd Paragraph) The "limma" package (version: 3.460) in R software was used to study the differentially methylation. (Please refer to Statistical analysis) The pipeline code for analyzing methylation sequencing is not available, please contact the corresponding author if request.	
If code is publicly available, provide accession number in repository, or DOI or URL.	R version 3.3.3 s limma" package (version: 3.460) (Please refer to Statistical analysis)	

Reporting

Adherence to community standards	Yes (indicate where provided: section/paragraph)	n/a

MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.		
State if relevant guidelines (eg., ICMJE, MIBBI, ARRIVE) have been followed, and whether a checklist (eg., CONSORT, PRISMA, ARRIVE) is provided with the manuscript.	ICMJE guidelines were followed, as the journal follows ICMJE recommendations for publication.	

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