The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

| Item | Recommendation | Section/line number, or reason for not reporting |
|------|----------------|--------------------------------------------------|
| **Study design** | 1 For each experiment, provide brief details of study design including: | Material and methods/line 173-174, line 184-186, line 217-218, line 302-303, line 324-326. In this study, two groups were used in animal environment 4-6 weeks in each. Referring to the types of experiments reported in the literature (Liu et al., 2017). |
| | a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. | |
| | b. The experimental unit (e.g. a single animal, litter, or cage of animals). | |
| **Sample size** | 2 a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. | In this study, the total number of animals used in the subcutaneous tumor bearing |
| | b. Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done. | |
| **Inclusion and exclusion criteria** | 3 a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established a priori. If no criteria were set, state this explicitly. | If the animals were successfully injected subcutaneously or via tail vein. In model of lung metastasis, one mice in the experimental and control groups. Material and methods/line 324-327. |
| | b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. | |
| | c. For each analysis, report the exact value of n in each experimental group. | |
| **Randomisation** | 4 a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. | 24 4-6-week-old male BALB/C N/J NJs mice, were obtained from the experimental animal center of Nanod Medical Institute, We took the following approach to minimize potential confounders. First, the same person handled |
| | b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly. | |
| **Blinding** | 5 Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis). | There are at least two persons to complete animal experiment, and the person who implements the treatment and the person |
| **Outcome measures** | 6 a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). | Material and methods/line 236-238, line 244-245, line 254-255, line 304-310. One of the main endpoints of this study was defined as the volume and weight of |
| | b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size. | |
| **Statistical methods** | 7 a. Provide details of the statistical methods used for each analysis, including software used. | Material and methods/line 316-328. |
| | b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met. | Material and methods/line 316-326. |
| **Experimental animals** | 8 a. Provide species-appropriate details of the animals used, including species, strain and substrate, sex, age or developmental stage, and, if relevant, weight. | Material and methods/line 296-299. |
| | b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures. | BALB/C N/J NJs mice purchased from ShanghaiSlpr 8K laboratory |
| **Experimental procedures** | 9 For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: | Material and methods/line 173-174, line 182-185, line 304-310. Mickey were housed in independent cages with free access to water and food. Referring to some types of experiments reported in the narrative of literature (Liu et al.) |
| | a. What was done, how it was done and what was used. | |
| | b. When and how often. | |
| | c. Where (including detail of any acclimatisation periods). | |
| | d. Why (provide rationale for procedures). | |
| **Results** | 10 For each experiment conducted, including independent replications, report: | Material and methods/line 316-328. |
| | a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). | Material and methods/line 316-328. |
| | b. If applicable, the effect size with a confidence interval. | |
The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

| Item                      | Recommendation                                                                 | Section/line number, or reason for not reporting |
|---------------------------|-------------------------------------------------------------------------------|-----------------------------------------------|
| **Abstract**              | 11 Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions. | Abstract, Results/line 70-79:line 11           |
| **Background**            | 12 a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach.  
   b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology. | Introduction/line 99-158; Nude mice do not reject cell or tissue transplants due to the inadequacy of immune suppression |
| **Objectives**            | 13 Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested. | Abstract, Results/line 63-91. |
| **Ethical statement**     | 14 Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification. | Material and methods/line 597-602. |
| **Housing and husbandry** | 15 Provide details of housing and husbandry conditions, including any environmental enrichment. | Same sex mice were housed to |
| **Animal care and monitoring** | 16 a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress.  
   b. Report any expected or unexpected adverse events.  
   c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this. | Before the mice were sacrificed, they were anesthetized intraperitoneally with ketamine/xylazine.  
Although it is based on experimental methods reported in the literature, in the mouse subcutaneous tumor bearing model, tumor sizes were measured with calipers. |
| **Interpretation/ scientific implications** | 17 a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.  
   b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results. | Discussion/line 497-571.  
Although our current research shows that miR-552-3p has tumorigenic effect in GBC, the clinical implications for the miR-552-3p promoter are not clear. |
| **Generalisability/ translation** | 18 Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate). | Our results show that miR-552-3p promotes GBC by inhibiting the expression of RG3A. This finding may have clinical indications for the future. |
| **Protocol registration** | 19 Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered. | N/A, our experiment does not involve this section. |
| **Data access**           | 20 Provide a statement describing if and where study data are available. | Footnote/line 587-589. |
| **Declaration of interests** | 21 a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated.  
   b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study. | Footnote/line 591-592.  
Acknowledgments, Contributions/line 574-583, line 51-57. |

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