EMBO *reports*

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Proteasomal Inhibition Selectively Kills Stem Cells in Glioma-Derived Cancer Cells Through Endoplasmic Reticulum-Associated Apoptosis

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EMBO PRESS

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Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

USEFUL LINKS FOR COMPLETING THIS FORM

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http://www.selectagents.gov/	List of Select Agents

B- Statistics and general methods

Please fill out these boxes (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	For all statistics, at least three independent experiments were performed to earn point value representing mean standard deviation. Statistical analysis were performed using two-sided, one type student T-TEST. p-value bigger than 0.05 was considered as significant value. For live cell counting experiments, at least 50 cells from randomly chosen fields were blindly counted. For colony formation assay, at least 50 colonies that are bigger than 100 μm from 4 randomely chosen fields were blindly counted.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For Figure 1G, six mice for each category were used for the experiments. For 100,000 GSCs, five mice were used finally. For Figure 8, four groups of mice have subcutaneously and orthotopically injections to establish tumors, respectively. For 500,000/mouse GSCs, forty mice were used finally. They are followed for a fixed amount of time and then are sacrificed.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	For cell culture studies, samples were not excluded.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	In animal experiments (Figure 8), mice were randomized into four groups after a few weeks, when tumor volume had reached approximately 180 mm3. For cell culture studies, cells from the same frozen stock batches cultured in large dishes, and distributed to small dishes and randomly allocated for the treatment.
For animal studies, include a statement about randomization even if no randomization was used.	In animal experiments (Figure 8), mice were randomized into four groups after a few weeks, when tumor volume had reached approximately 180 mm3.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	For colony formation assay, Colonies bigger than 100 μm in diameter were blind-counted from four microscopic fields. Three independent experiments were performed. For live cell counting, at least 50 cells from randomly chosen fields were blindly counted. Three independent experiments were performed.
4.b. For animal studies, include a statement about blinding even if no blinding was done	N/A
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes. We specified the test used to calculate p—values in the respective figure legends (page 24, 25, 27, 29). One week following implantation, animals received 50 mg/kg D-luciferin, and tumor implantation was verified by luciferase bioluminescence.
Is there an estimate of variation within each group of data?	Mean standard deviation were calculated from three independent experiments. An estimate of variation for each factor (groups) was also calculated by the multiple replicate 2-factor ANOVA.
Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	PARP1 : PLA0184 (sigma), Ubiquitin : SAB4503053 (sigma), NFκBp65 : SAB1305640 (sigma), NFκBp50 : sc-1190 (sigma), IκB-α : 10505 (sigma), IκB-β : PRS4243 (sigma), IκB-ε : 10632 (sigma), Actin: A-5441 (sigma), Nestin : AB5922 (Merck), Musashi : Ab-5977(Merck), SOX2 :AF2018 (R&D Systems), GFAP : Z0334 (Dakocytomation), βIII-tubulin : MMS-435P (Covance), caspase 3 : 9662 (Cell Signaling Technology; CST), IKK-α : 2682 (CST), IKK-β : 2684 (CST), JNK : 9252 (CST), p-JNK : 9251 (CST), p38 : 9212 (CST), p-p38 : 9211 (CST), USP14 : A300-919 (Bethyl Laboratory), 19S RPT5 : BML-PW8770 (ENZO Life), 20S α3 : BML-PW8115 (ENZO Life), 20S α7 : BML-PW8110 (ENZO Life), RNF123 : Ab57549 (Abcam), UBR2 : NBP1-45243 (Novus Biologicals)
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Glioma Stem cell lines are obtained from University of Pittsburgh. We confirmed that mycoplasma contamination using Plasmocin reagents (InvivoGen). The references are cited in the main text.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Five-week-old female BALB/c nude mice were obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan) and housed in a specific pathogen-free environment. Animals were kept in IVC cages in a temperature-controlled environment with a 12 hours dark/light cycle and fed with standard plant based chow.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Animal studies were conducted according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised in 1996) and the protocols (12-0304) approved by the Institutional Animal Care and Use Committee at Seoul National University.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Compliance was confirmed.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	The data in GEOArchive files were deposited in the Gene Expression Omnibus (GEO) of NCBI (http://www.ncbi.nlm.nih.gov/geo/) under the accession number of GSE62356.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4026 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	N/A
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	N/A