# ***Supplementary information***

**Title:** Mitotic catastrophe is a putative mechanism underlying the weak correlation between sensitivity to carbon ions and cisplatin

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**Supplementary Table 1.** Summary of the studies on synergistic cell killing effect between X-rays or carbon ions and cisplatin in human cancer cells.

| Radiation | Cell line | Synergistic? | References |
|---|---|---|---|
| X-rays | AE7 | yes | 11 |
| | AN3 | yes | |
| | ECC1 | yes | |
| | HEC1A | yes | |
| | HEC1B | yes | |
| | SKUT1B | yes | |
| | HeLa | yes | 12 |
| | C33A | yes | |
| | A549 | yes | 13 |
| | CaSki | yes | |
| | SW-1573 | yes | 14 |
| | WiDr | yes | 8 |
| | A2780cp | yes | 15 |
| | A2780 | no | |
| | DU-145 | controversial | 16 |
| | 19 primary tumors | no | 17 |
| | HeLa | no | 18 |
| | CaSki | no | |
| | MRI-186 | no | |
| | LX-I | no | |
| | A549 | no | 19 |
| | HepG2 | no | 20 |
| | OV-1063 | no | 21 |
| | TE-2 | no | 9 |
| | U87 | no | 10 |
| Carbon ion | MDA-MB-231 | yes | 7 |
| | WiDr | no | 8 |
| | TE-2 | no | 9 |
| | U87 | no | 10 |

See "Supplementary Methods" for the detailed methods for litereature search.

**Supplementary Table 2.** Parameters for X-ray and carbon ion sensitivity.

| Cell line | X-rays | | | | | | Carbon ion | | | | | | RBE | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D10 | | α | | β | | D10 | | α | | β | | | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| H1299 | 10.9 | 0.76 | 0.0024 | 0.026 | 0.019 | 0.0019 | 3.9 | 0.12 | 0.079 | 0.034 | 0.13 | 0.018 | 2.8 | 0.075 |
| H157 | 6.7 | 0.27 | 0.051 | 0.079 | 0.043 | 0.010 | 3.3 | 0.14 | 0.63 | 0.060 | 0.020 | 0.022 | 2.0 | 0.13 |
| LK2 | 6.7 | 0.28 | 0.066 | 0.031 | 0.041 | 0.0049 | 3.3 | 0.075 | 0.40 | 0.095 | 0.094 | 0.029 | 2.1 | 0.042 |
| H1703 | 9.2 | 0.66 | 0.16 | 0.022 | 0.010 | 0.0033 | 4.3 | 0.37 | 0.31 | 0.12 | 0.055 | 0.033 | 2.1 | 0.21 |
| HCT15 | 6.3 | 0.11 | 0.13 | 0.017 | 0.039 | 0.0029 | 2.3 | 0.061 | 0.75 | 0.15 | 0.10 | 0.028 | 2.7 | 0.17 |
| II-18 | 7.4 | 0.78 | 0.048 | 0.26 | 0.036 | 0.035 | 4.1 | 0.56 | 0.42 | 0.062 | 0.035 | 0.0069 | 1.8 | 0.21 |
| A549 | 6.6 | 0.67 | 0.30 | 0.11 | 0.0076 | 0.016 | 4.0 | 0.21 | 0.25 | 0.079 | 0.084 | 0.020 | 1.7 | 0.036 |
| WiDr | 6.9 | 0.11 | 0.0047 | 0.031 | 0.048 | 0.0051 | 3.3 | 0.10 | 0.33 | 0.25 | 0.12 | 0.084 | 2.1 | 0.12 |
| HCT116 | 4.0 | 0.13 | 0.39 | 0.15 | 0.044 | 0.035 | 1.6 | 0.19 | 1.3 | 0.18 | 0.088 | 0.048 | 2.6 | 0.26 |
| A427 | 5.3 | 0.43 | 0.29 | 0.049 | 0.028 | 0.010 | 2.1 | 0.14 | 1.0 | 0.21 | 0.033 | 0.067 | 2.6 | 0.23 |
| KYSE70 | 6.8 | 0.26 | -0.018 | 0.085 | 0.053 | 0.019 | 2.5 | 0.10 | -0.33 | 0.11 | 0.51 | 0.055 | 2.8 | 0.19 |
| H1975 | 4.7 | 0.26 | 0.44 | 0.070 | 0.010 | 0.0088 | 2.4 | 0.26 | 0.20 | 0.21 | 0.32 | 0.079 | 2.0 | 0.15 |
| HCC827 | 5.5 | 0.22 | 0.29 | 0.071 | 0.024 | 0.012 | 2.6 | 0.064 | 0.60 | 0.10 | 0.11 | 0.029 | 2.1 | 0.124 |
| H522 | 6.0 | 0.29 | 0.11 | 0.063 | 0.046 | 0.012 | 2.1 | 0.08 | 0.97 | 0.036 | 0.076 | 0.0049 | 2.9 | 0.11 |
| TE2 | 2.1 | 0.10 | 0.88 | 0.13 | 0.093 | 0.047 | 0.94 | 0.13 | 1.5 | 0.56 | 1.1 | 0.85 | 2.3 | 0.25 |
| H460 | 4.8 | 0.20 | 0.23 | 0.017 | 0.052 | 0.0038 | 1.8 | 0.061 | 0.82 | 0.11 | 0.25 | 0.078 | 2.6 | 0.079 |
| HeLa | 6.7 | 0.19 | 0.19 | 0.080 | 0.023 | 0.014 | 3.1 | 0.079 | 0.26 | 0.19 | 0.15 | 0.065 | 2.1 | 0.10 |
| H1650 | 4.8 | 1.00 | -0.10 | 0.20 | 0.12 | 0.037 | 2.7 | 0.16 | 0.56 | 0.10 | 0.10 | 0.024 | 1.8 | 0.17 |
| Ma-24 | 3.9 | 0.35 | 0.22 | 0.27 | 0.11 | 0.089 | 2.8 | 0.14 | 0.34 | 0.15 | 0.17 | 0.056 | 1.4 | 0.29 |
| PC9 | 3.4 | 0.13 | 0.64 | 0.14 | 0.011 | 0.026 | 2.5 | 0.19 | 0.32 | 0.28 | 0.25 | 0.12 | 1.4 | 0.18 |

RBE, relative biological effectiveness; SD, standard deviation.

**Supplementary Table 3.** Correlation between X-ray- and cisplatin-sensitivity.

| Index | X-rays | Cisplatin | | |
|---|---|---|---|---|
| | | 0.2 μM | 1 μM | IC50 |
| R value | SF2Gy | 0.59 | 0.63 | 0.68 |
| | SF4Gy | 0.70 | 0.76 | 0.76 |
| | SF6Gy | 0.62 | 0.65 | 0.61 |
| | SF8Gy | 0.41 | 0.68 | 0.60 |
| | D10 | 0.70 | 0.76 | 0.74 |
| P value | SF2Gy | 6.0E-3 | 2.7E-3 | 9.6E-4 |
| | SF4Gy | 4.1E-4 | 2.0E-7 | 2.0E-7 |
| | SF6Gy | 4.5E-3 | 2.8E-3 | 5.5E-3 |
| | SF8Gy | 0.035 | 1.4E-3 | 6.2E-3 |
| | D10 | 4.6E-4 | 2.0E-7 | 8.1E-5 |

SFXGy indicates the surviving fraction at X Gy.

**Supplementary Table 4.** Correlation between carbon ion- and cisplatin-sensitivity.

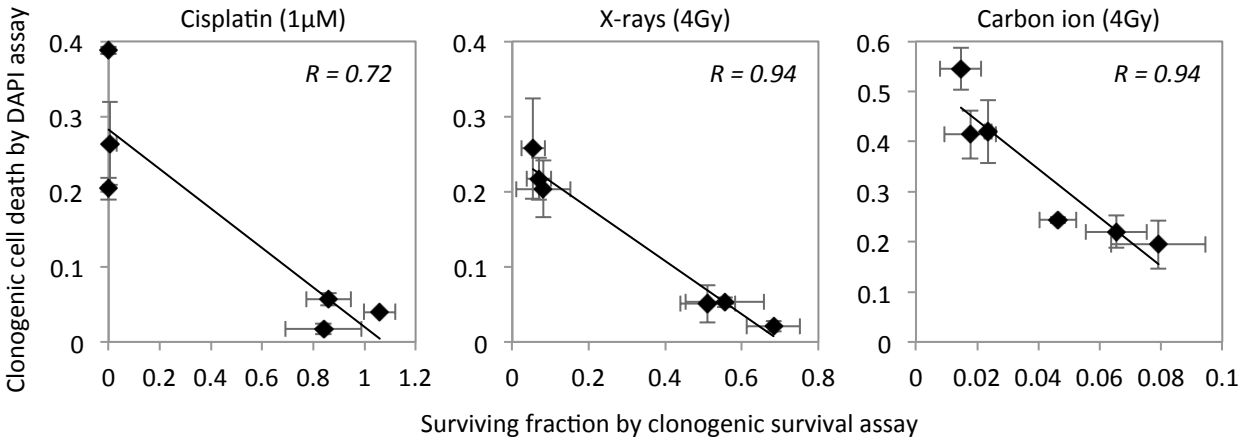
| Index | Carbon ion | Cisplatin | | |
|---|---|---|---|---|
| | | 0.2 μM | 1 μM | IC50 |
| R value | SF1Gy | 0.44 | 0.47 | 0.46 |
| | SF2Gy | 0.35 | 0.43 | 0.39 |
| | SF3Gy | 0.30 | 0.55 | 0.53 |
| | SF4Gy | 0.51 | 0.53 | 0.44 |
| | D10 | 0.45 | 0.57 | 0.49 |
| P value | SF1Gy | 0.049 | 0.037 | 0.042 |
| | SF2Gy | 0.13 | 0.056 | 0.089 |
| | SF3Gy | 0.23 | 0.018 | 0.024 |
| | SF4Gy | 0.031 | 0.024 | 0.068 |
| | D10 | 0.044 | 9.2E-3 | 0.027 |

SFXGy indicates the surviving fraction at X Gy.

**Supplementary Table 5.** Cell lines used in the present study.

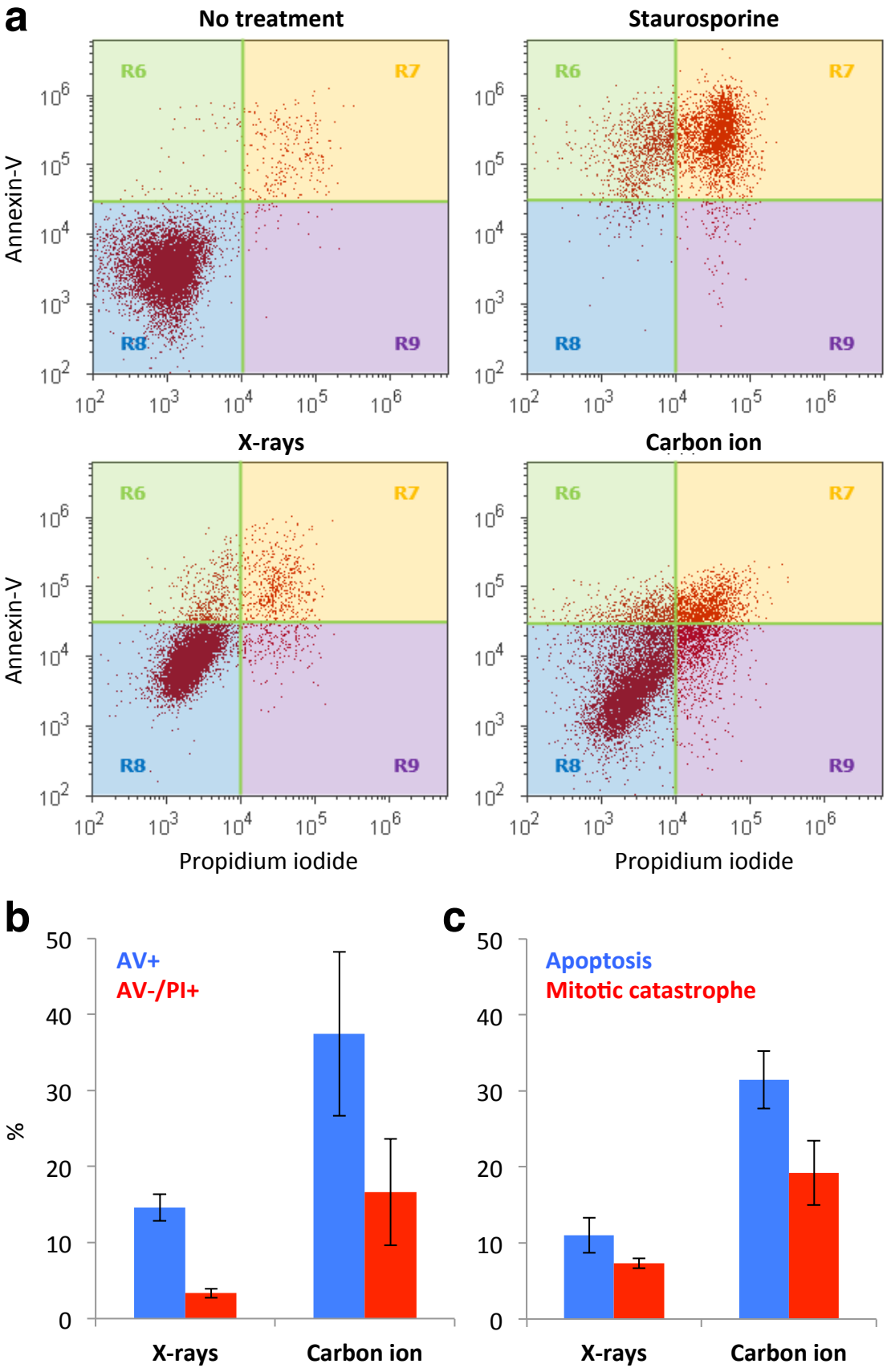
| Cell line | Cancer type | Histology | Obtained from |
|---|---|---|---|
| KYSE70 | Esophageal cancer | Squamous cell carcinoma | Health Science Research Resources Bank (Osaka, Japan) |
| TE2 | Esophageal cancer | Squamous cell carcinoma | University of Tohoku Cell Bank (Sendai, Japan) |
| A427 | Non-small cell lung carcinoma | Adenocarcinoma | ATCC (Manassas, VA, USA) |
| A549 | Non-small cell lung carcinoma | Adenocarcinoma | ATCC (Manassas, VA, USA) |
| H522 | Non-small cell lung carcinoma | Adenocarcinoma | ATCC (Manassas, VA, USA) |
| H1650 | Non-small cell lung carcinoma | Adenocarcinoma | ATCC (Manassas, VA, USA) |
| H1703 | Non-small cell lung carcinoma | Adenocarcinoma | ATCC (Manassas, VA, USA) |
| H1975 | Non-small cell lung carcinoma | Adenocarcinoma | ATCC (Manassas, VA, USA) |
| HCC827 | Non-small cell lung carcinoma | Adenocarcinoma | ATCC (Manassas, VA, USA) |
| II-18 | Non-small cell lung carcinoma | Adenocarcinoma | JCRB Cell Bank (Osaka, Japan) |
| Ma-24 | Non-small cell lung carcinoma | Adenocarcinoma | Dr. Shimizu (Tokushima University, Tokushima, Japan) |
| PC9 | Non-small cell lung carcinoma | Adenocarcinoma | Dr. Kato (Tokyo Medical Collage, Tokyo, Japan) |
| H157 | Non-small cell lung carcinoma | Squamous cell carcinoma | Dr. Harris (National Institute of Health, MD, USA) |
| LK2 | Non-small cell lung carcinoma | Squamous cell carcinoma | JCRB Cell Bank (Osaka, Japan) |
| H460 | Non-small cell lung carcinoma | Large cell carcinoma | ATCC (Manassas, VA, USA) |
| H1299 | Non-small cell lung carcinoma | Large cell carcinoma | ATCC (Manassas, VA, USA) |
| HCT15 | Colorectal cancer | Adenocarcinoma | ATCC (Manassas, VA, USA) |
| HCT116 | Colorectal cancer | Adenocarcinoma | Dr. Vogelstein (Johns Hopkins University, MD, USA) |
| WiDr | Colorectal cancer | Adenocarcinoma | ATCC (Manassas, VA, USA) |
| HeLa | Uterine cervical cancer | Adenocarcinoma | ATCC (Manassas, VA, USA) |

# Supplementary Figure 1




**Supplementary Figure 1.** Correlation between the total amount clonogenic cell death (apoptosis, mitotic catastrophe and senescence) as assessed by the DAPI staining assay and the surviving fraction as assessed by the clonogenic survival assays in PC9, Ma-24, H1650, H1299, H157, and LK2 cells, as assessed by Spearman Rank Order test. Note that the data for the clonogenic survival assay and those for the DAPI staining assay are the same as in Figure 1 and 2, and Figure 4b, respectively, but now in the different context.

# Supplementary Figure 2




**Supplementary Figure 2.** Cell death induced by X-ray or carbon ion irradiation as assessed by flow cytometry or the DAPI staining assay. PC9 cells were treated with X-rays (4 Gy for 72 h), carbon ions (4 Gy for 72 h), or staurosporine (1 μM for 24 h), followed by the staining with AV and PI or DAPI. The AV/PI-stained cells were analyzed by flow cytometry, while the DAPI stained cells were analyzed for nuclear morphologies representing apoptosis and mitotic catastrophe (see "Materials and Methods" for definitions). **(a)** Representative flow cytometry plots. High AV-positivity in staurosporine-treated cells indicates the robustness of the assay (Supplementary references 1, 2). **(b)** Percentages of AV-positive and AV-negative/PI-positive cells determined by flow cytometry. **(c)** Percentages of apoptosis and mitotic catastrophe determined by the DAPI staining assay. Note that the data for the DAPI staining assay are the same as in Figure 4b but now in the different context.

## Supplementary Methods

*Literature search: synergistic cell killing by X-rays or carbon ions plus cisplatin.*

On January 15, 2016, two authors (D. K. and T. Oike) performed independent literature searches in PubMed using the search term "cisplatin AND radiosensitization" and obtained 147 hits. A full text screen for all available papers identified 15 that used clonogenic survival assays to examine the cell killing effects of X-rays and/or carbon ions combined with cisplatin in human cancer cells. The presence or absence of synergistic cell killing effect was analyzed: cell killing effects classified as "supra-additive", "independently toxic", and "radiosensitizing" were considered synergistic, whereas effects described as "additive" were considered non-synergistic. The results are summarized in Supplementary Table 1.

## Supplementary References


1. Nakagawa, T. *et al*. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. *Nature* **403**, 98–103 (2000)
2. Takayama, S. *et al*. Cloning and functional analysis of BAG-1: a novel Bcl-2-binding protein with anti-cell death activity. *Cell* **80**, 279–284 (1995)