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# Proliferation impairment of chondrosarcoma cells irradiated with X-Rays and carbon-ion beams

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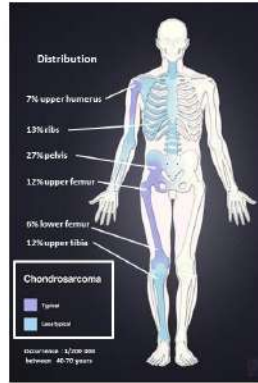
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Chondrosarcoma is a malignant tumor arising from cartilaginous tissues, described as radiation- and chemo-resistant to conventional treatments.

The primary treatment consists in wide surgical resection, which may lead to severe disabilities; in addition, this procedure is not feasible for some inoperable locations such as skull base chondrosarcoma.

Carbon-ion irradiation (hadron-therapy) has been successfully used in the treatment of chondrosarcoma, due notably to a higher biological effectiveness and a better ballistic as compared with conventional radiotherapy with X-Rays.



### Aim of this study

- explore the differential cellular responses of chondrosarcomas to conventional radiotherapy and hadrontherapy
- understand the biological effects of carbon beams in cancer treatment.
- estimate the capacity of PARP-1 inhibitor in radio-sensitizing chondrosarcoma cells

### Irradiation methods

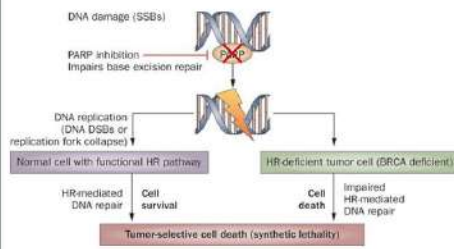
- Four human chondrosarcoma cell lines of different grades and displaying differential radio-sensitivities, were irradiated (1-2 Gy/min) with:
- X-Rays (225 kV)
  - Carbon ions (LET 33 KeV/μm)
  - Carbon / Neon / Oxygen ions (LET ~ 85 KeV/μm)
  - with / without PARP inhibitors : Olaparib (clinical trial in phase II) and Rucaparib (phase I)

### Irradiation Facilities



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### Synthetic lethality between BRCA deficiency and PARP inhibition



DNA is subject to frequent damage, but SSBs are normally efficiently repaired by BER. PARP is a key component of BER and PARP inhibition leads, therefore, to persistence of SSBs. A replication fork may encounter a persistent SSB during DNA replication, which causes the replication fork to collapse or the formation of a DSB.

These breaks are normally repaired by the homologous recombination DSB repair pathway, which requires BRCA1 and BRCA2.

In the absence of functional BRCA1 or BRCA2, for example through mutation, the DNA cannot be repaired, or is repaired by alternative pathways that are highly error prone, which results in gross genomic instability and cell death.

**Abbreviations:**  
BER, base excision repair; DSB, double strand break; HR, homologous recombination; PARP, poly(ADP-ribose)polymerase; SSB, single strand break.

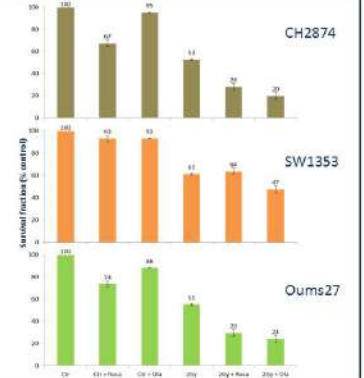
Boreyko, S. et al. (2010) Making the best of PARP inhibitors in ovarian cancer. *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2010.115

### Radio-sensitizing of chondrosarcoma cells with PARP inhibitors

Rucaparib (Ruc) and Olaparib (Ola) were added in cell culture at a final concentration of 0.4 μM and 2 μM, respectively. Both PARP inhibitors were introduced 2 h before irradiation, and removed 16 h after irradiation. Cells were irradiated at 2Gy with X-rays and were left for 16 h post irradiation, then trypsinized for subsequent clonogenic assays.

⇒ This preliminary study [1 exp] showed a differential radio-sensitizing effect of PARP inhibitors with the three chondrosarcoma cell-lines analysed.

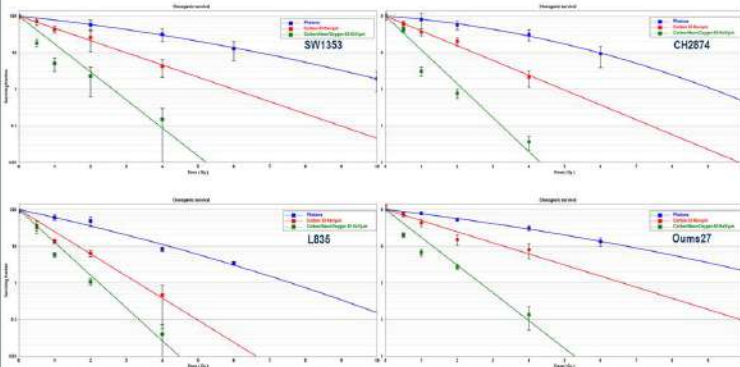
The highest differential effect is obtained with CH2874 cells (% 2Gy+Ola/2Gy) : CH2874 (62%) - Oums27 (56%) - SW1353 (23%)



### Clonogenic Forming Efficiency assays

Cells were irradiated at confluency with X-Rays and ion beams (33 KeV/μm and 85 KeV/μm) at different doses. Following a 18h incubation time, cells were seeded in 6 wells plates at low density. Colonies were stained with violet crystal after 10 to 12 days and clones (>50 cells) were counted.

Multiple biological repetitions were used to plot the results.

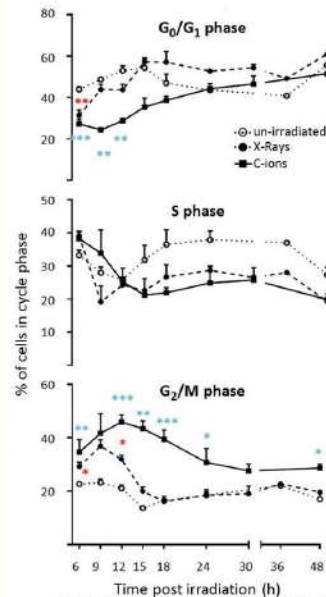


⇒ A differential radio-sensitivity is observed as a function of:

- irradiation quality : RBE = 2,2 - 2,5 (33 KeV/μm)  
RBE = 3,74 - 5,51 (85 KeV/μm)
- chondrosarcoma cell line : Oums27 < SW1353 < CH2874 < L835 (X-Rays and 33 KeV/μm)  
Oums27 < SW1353 < L835 < CH2874 (85 KeV/μm)  
most resistant - < - most sensible

### Cell cycle analysis of SW1353 cells

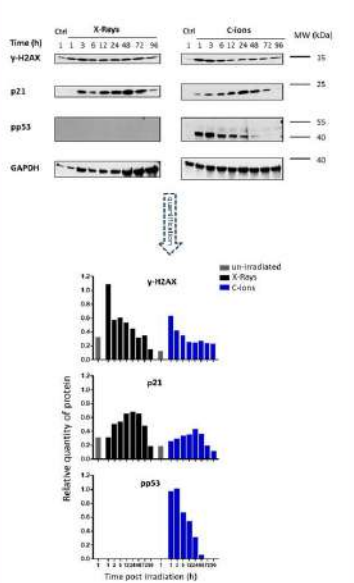
Following irradiation (X-Rays and C-ions 33 KeV/μm), SW1353 cells were analyzed by flow cytometry to estimate the percentage of cells in each cycle phase



⇒ C-ions induced a longer G2/M phase arrest as compared with X-Rays, showing the difficulty of cells to repair DNA damages following C-ion irradiation.

### Western blotting analysis of SW1353 cells

Following irradiation (X-Rays and C-ions 33 KeV/μm), SW1353 cells were analyzed by western blotting to estimate the relative amount of specific proteins markers of irradiation responses



⇒ Both irradiations activated γ-H2AX and P21 and C-ions induced specifically a higher amount of phospho-P53, in agreement with a longer arrest observed by cell cycle analysis.