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Radio-sensitization of chondrosarcoma cells by different PARP-inhibitors

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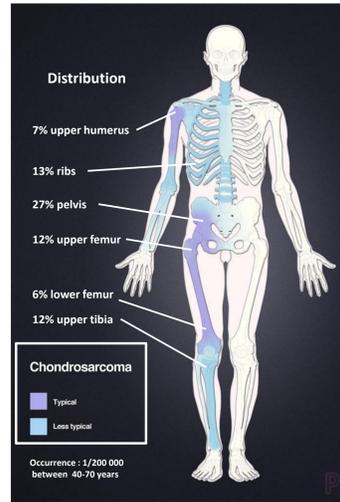
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Chondrosarcoma is a malignant tumor arising from cartilaginous tissues, described as radiation- and chemo- resistant to conventional treatments.

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.

Proton and Carbon-ion irradiations (hadron-therapy) have 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.

The use of **PARP inhibitors** can increase the potential of these irradiations to treat such radio-resistant cancer, prevent metastasis and secondary cancers.



Irradiations / treatments

Three human chondrosarcoma cell lines of different grades (SW1353 GII, CH2879 GIII and OUMS27 GIII)

- were irradiated with **X-Rays** (X-Rad 225 Cx), **Proton** and **Carbon ions** (when mentioned, in presence of Temozolomide (TMZ : induces guanine alkylation))
- with / without PARP inhibitor : **Olaparib (Ola)**, **AG14361 (AG)**.

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 inhibitors in radio-sensitizing chondrosarcoma cells

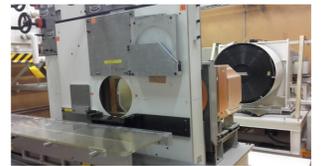
Irradiation facilities with accelerated ions



INFN, Catania, Italy
Protons / Carbon ions

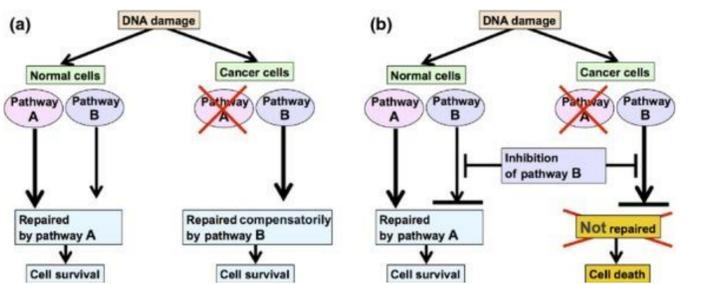


GANIL, Caen, France
Carbon ions



NIRS, Chiba, Japan
Carbon ions

Principle of synthetic lethality



DNA damage is often processed by multiple DNA repair pathways. In the example shown here, pathways A and B are both intact in normal cells, whereas pathway A is defective in cancer cells.

- In the absence of the pathway B inhibitor, cancer cells can survive, because the defect in pathway A is compensated by the alternative pathway B.
- When the cells are treated with the pathway B inhibitor, both pathways will be blocked in cancer cells, which will result in cell death. However, normal cells will not be affected, because inhibition of pathway B will be compensated by pathway A.

Mutation profiles of chondrosarcoma cell lines

	SW1353		CH2879		OUMS27	
ATM			substitution	missense	substitution	missense
ATR			substitution	missense		
BABAM1			substitution	missense		
CDK12					substitution	missense
CHEK1	substitution	missense	substitution	missense	substitution	missense
ERCC1	substitution	missense				
FANCA			substitution	missense		
FANCD2	substitution	missense				
FANCG	substitution	missense				
FANCM	substitution	missense				
HDAC2			deletion	Frameshift		
NBN			substitution	nonsense		
PARP1					substitution	missense
PTEN	substitution	missense	substitution	missense		
RAD50			deletion	Frameshift		
RAD51D	substitution	missense				
SLX4	substitution	missense			substitution	missense
SMARCA4						
TOPBP1	substitution	missense				
UIMC1	substitution	missense				
XRCC4	substitution	missense	substitution	missense		

A panel of genes (69), implicated in DNA repair, have been sequenced for each cell line. An illumina NextSeq, 2x75pb paired-end technic was used. Bioinformatics analysis was performed HaplotypeCaller (Broad Institute).

All cell lines present mutations on genes implicated in DNA repair, and/or DNA stability. These mutation could have an impact on cell survival when DNA repair is partially impaired using PARPi.

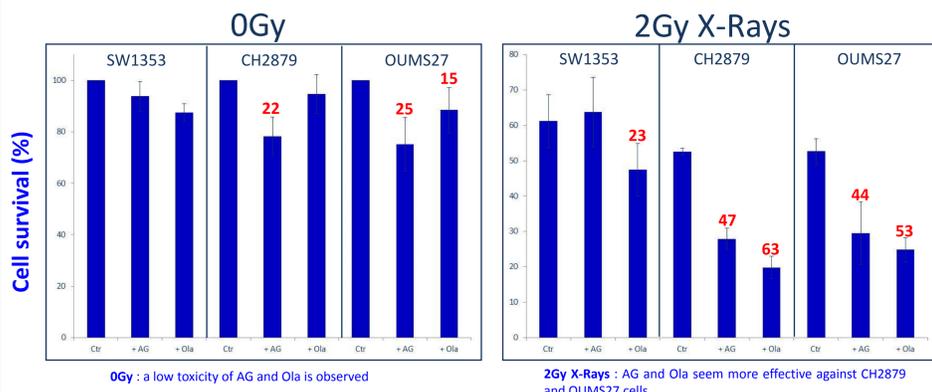
Mutations in bold correspond to homozygous genotype, the others are heterozygous.

CHEK1 gene seems to be mutated in both cell lines, whereas the other mutations are highly dependent of the cell line considered. Such mutation status can impact cell survival especially following irradiation and drug treatment (PARPi +/- TMZ)

Clonogenic cell survival assays

Cells were irradiated at confluency with X-Rays and ion beams at the corresponding physical doses. Following a 18H incubation time, cells were seeded at low density. Colonies were stained after 10 to 12 days and clones (>50 cells) were counted.

In red = % sensitization effect vs corresponding control (when significant p<0,05)
(multiple biological repetitions were used to plot the results)



Cell cycle analysis

Following irradiation (C-ions), OUMS27 cells were analyzed by flow cytometry to estimate the percentage of cells in each cycle phase

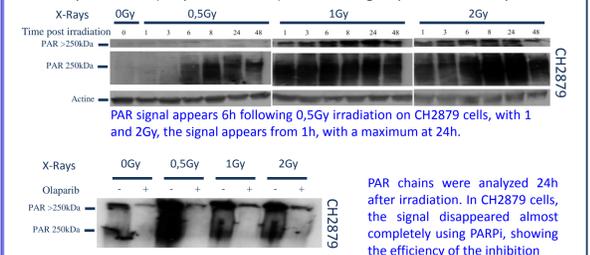
	G0G1	S	G2/M
0Gy	50.53	11.80	16.10
0Gy + Ola	42.56	13.83	23.43
0Gy + TMZ	51.95	12.73	13.47
0Gy + Ola + TMZ	32.51	13.42	23.81
1Gy	51.90	13.05	15.34
1Gy + Ola	39.38	11.63	20.21
1Gy + TMZ	50.17	11.83	15.24
1Gy + Ola + TMZ	31.57	15.93	28.73
2 Gy	47.13	10.88	16.68
2Gy + Ola	43.14	11.80	21.23
2Gy + TMZ	47.91	12.05	12.97
2Gy + Ola + TMZ	26.86	12.72	29.08

Only PARP inhibition can induce S-phase delay and G2/M arrest (red data) with OUMS27 cells

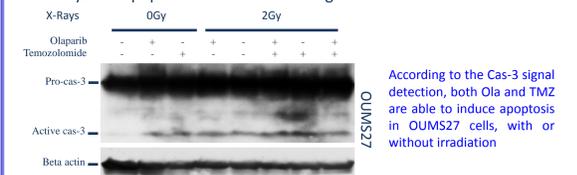
The combination Ola + TMZ effectively arrests cells in G2/M phase, with a greater intensity than 1Gy or 2Gy C-ions alone.

Western blotting analysis

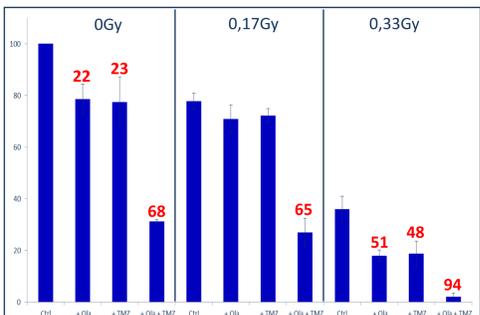
• Analysis of PAR (Poly-ADP Ribose) chains using a specific antibody



• Analysis of apoptosis induction following irradiation



Low dose C-ions and TMZ (CH2879)



C-ions and TMZ (OUMS27)

