Heavy Ion Therapy Research Integration

Specialised Course on Heavy Ion Therapy Research

4–8 Jul 2022

Online

Radiobiology for Hadron Therapy

ANGELICA FACOETTI, PhD

CNAO - National Center for Oncological Hadrontherapy

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101008548

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***Classic* radiation biology for hadrontherapy**

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- LET
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***Rock* radiation biology for hadrontherapy**

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Migration-invasion

Angiogenesis

Cell differentiation

Immunogenic properties

Cancer stem cells

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Radiobiology

- Molecular radiobiology
- Normal tissue radiobiology
- Cell radiobiology
- Clinical radiobiology
- DNA radiobiology
- Physics radiobiology
- Low-dose radiobiology
- Heavy-ion radiobiology
- Translational radiobiology
- Chemical radiobiology
- Applied radiobiology
- Computational radiobiology
- …

→ **Disclaimer**

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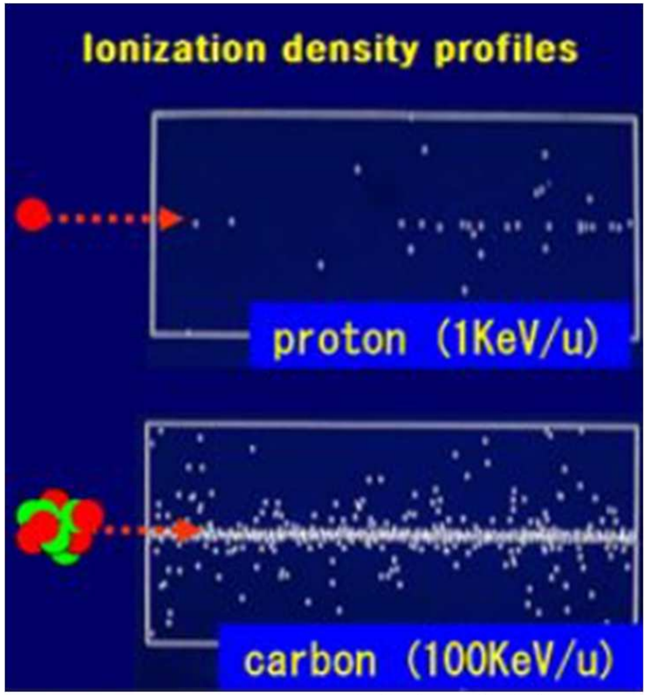
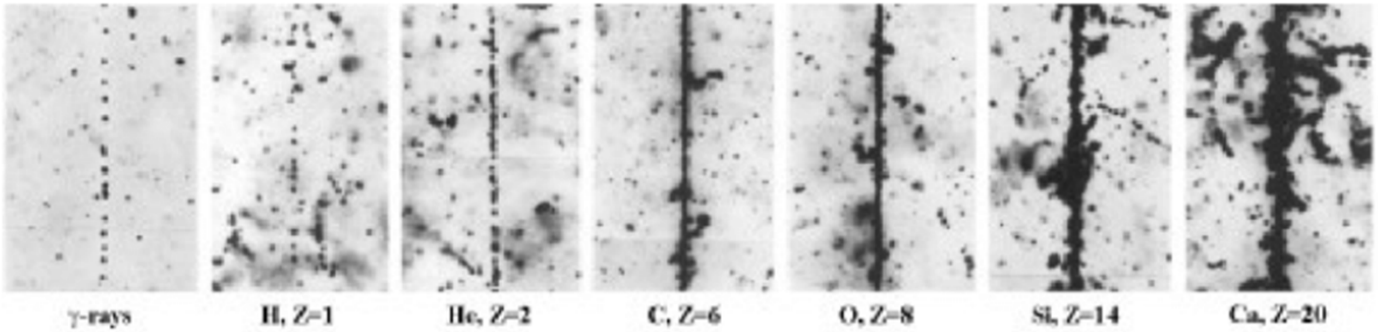
LET – Linear Energy Transfer

It is the amount of energy that an ionizing particle transfers to the material traversed per unit distance and describes the pattern of energy deposition within particle track



Mi-Sook Kim,

LET= the energy deposited per unit track (KeV/µm)



Tracks in photo-emulsions of electrons produced by γ-rays and tracks of different nuclei of the primary cosmic radiation moving at relativistic velocities

Baumstark-Khan, Christa & Facius, R, 2002.

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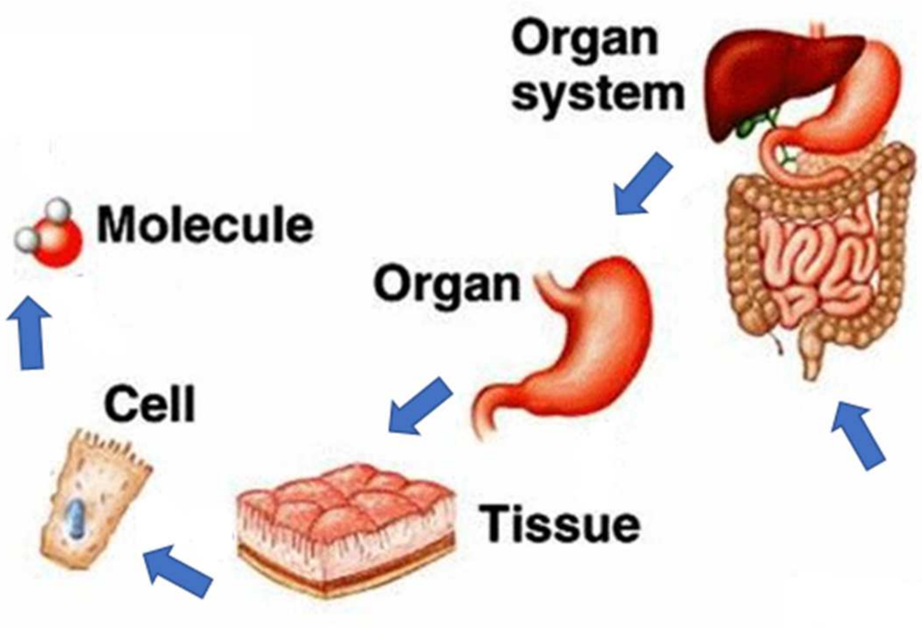
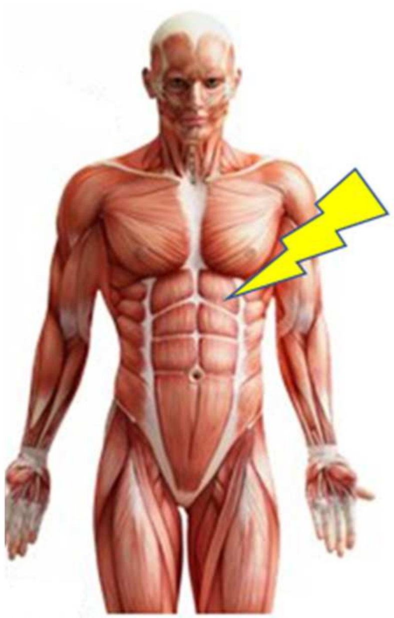
LET – Linear Energy Transfer

It is the amount of energy that an ionizing particle transfers to the material traversed per unit distance and describes the pattern of energy deposition within particle track

It describes the action of radiation into "matter".





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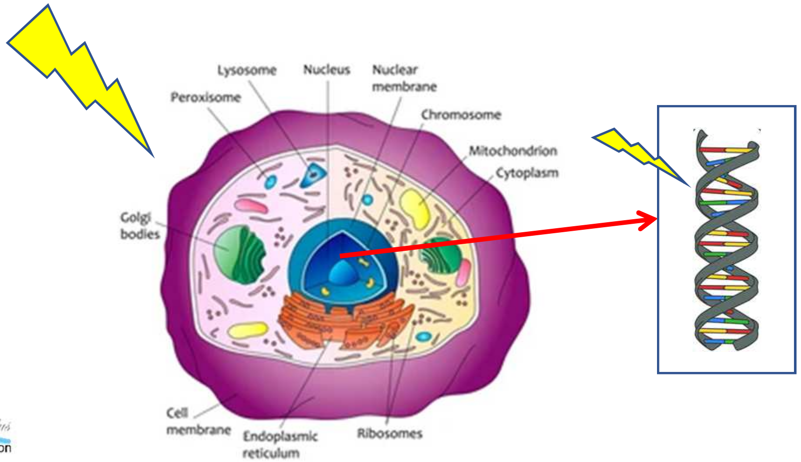
«Classic» radiation biology for hadrontherapy

Radiation causes damage to all cellular molecules, but <u>DNA damage</u> is most critical (most cellular and molecular components can be replaced)



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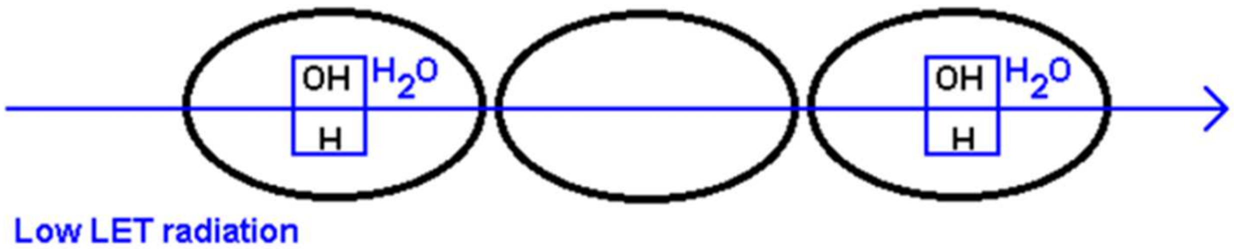
Low *versus* High LET radiations

High LET radiation ionizes water into hydrogen atoms and hydroxyl radicals over a very short track. There is so high probability that two events occur in a single cell so as to form a pair of adjacent OH radiacal that recombine to **form peroxide, H_2O_2**, which can produce oxidative damage to the cell



Low LET radiation also ionizes water molecules but over a much longer track, so most likely two events occur in separate cells, such that adjacent radicals are of the opposite type: the H and OH radicals reunite and **reform H_2O**

DrAyush Garg

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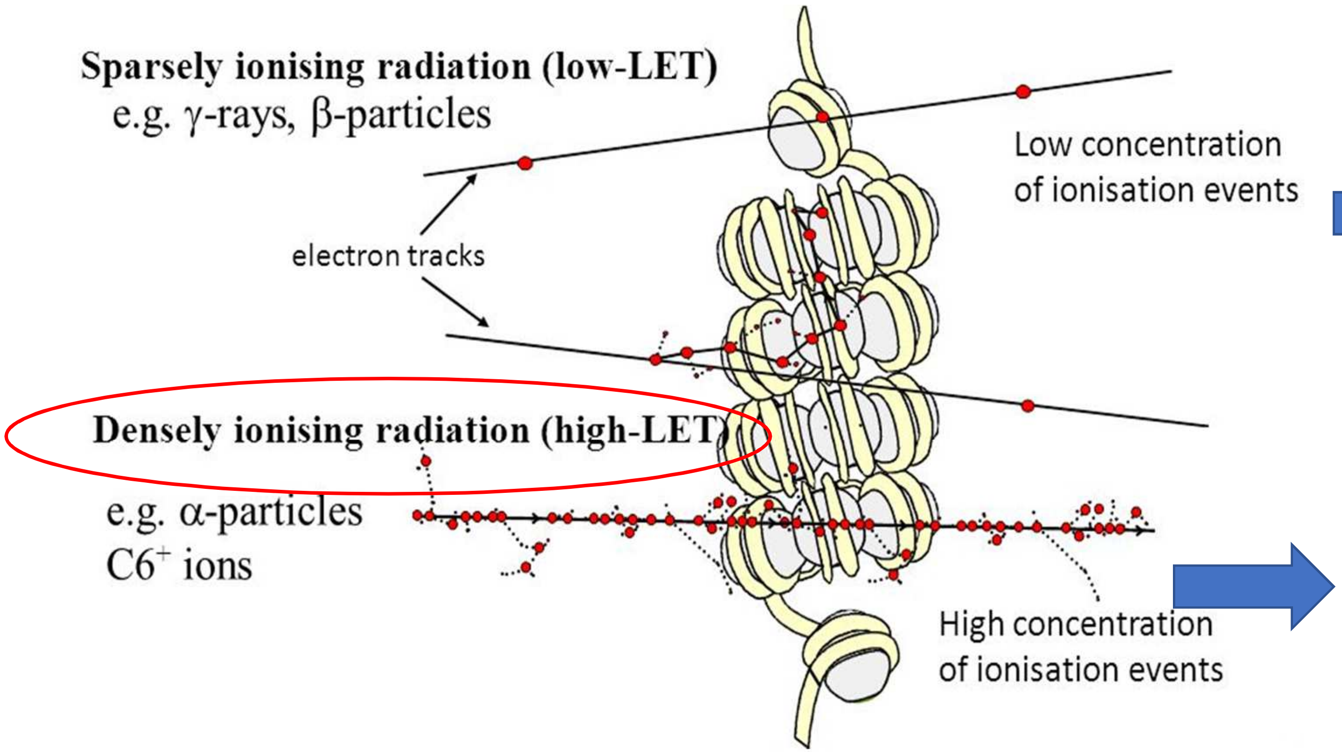
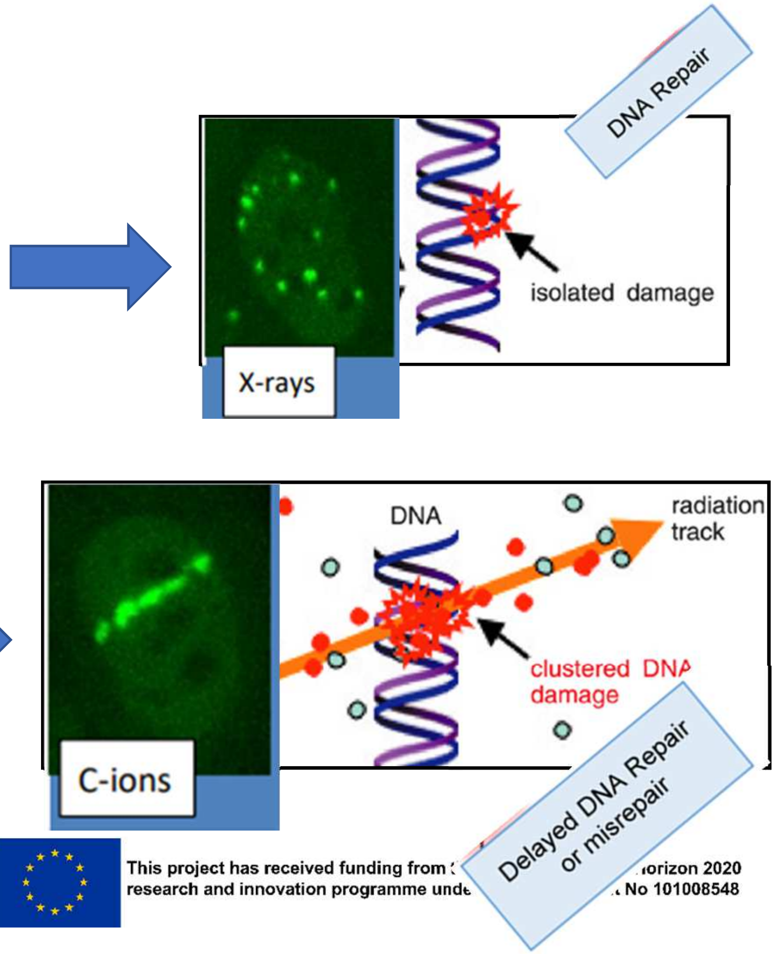
Low *versus* High LET radiations on the DNA





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For biological radiation effects the efficiency of a radiation type increases as the ion density along the tracks increases

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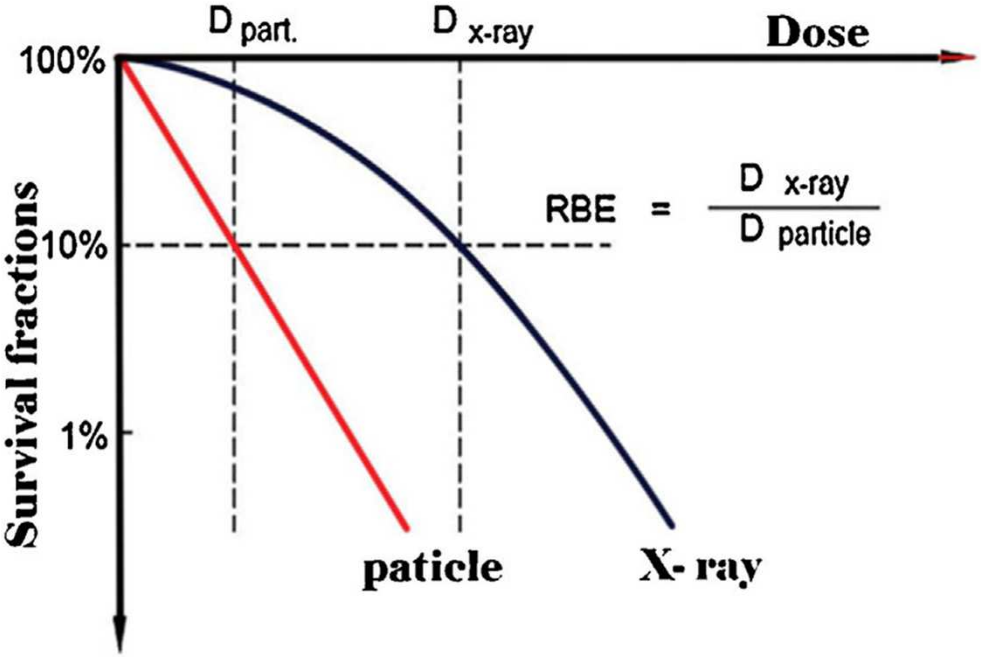
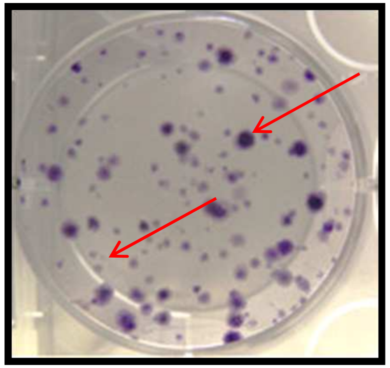
research and innovation programme unde… No 101008548

RBE - Relative biological effectiveness





Relative biologic effectiveness (RBE) is used to describe the biologic effectiveness of radiation of different qualities.

N.B: Protons have an **empirical RBE of 1.1**, which is only slightly greater than that of x-rays. Thus, the biologic effects of protons and photons are considered similar.

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BUT…factors that determine RBE

- Radiation quality (Type of radiation, Energy of radiation…)
- Dose rate
- Radiation Dose
- Number of dose fractions
- Biologic system
- Endpoint
- …
- Experimental conditions

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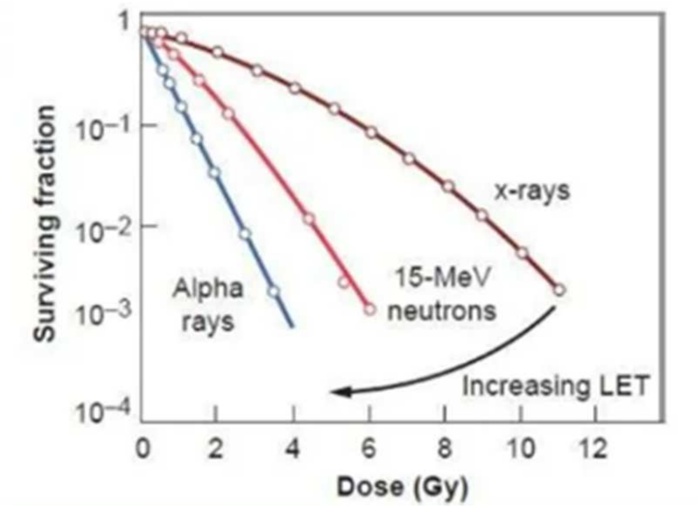
RBE as a function of LET



As the LET increases from about 2keV/µm for x-rays up to 150 keV/ µm for alpha particles, the survival curve becomes steeper and the shoulder of the curve becomes progressively smaller.

Higher the LET higher the RBE???

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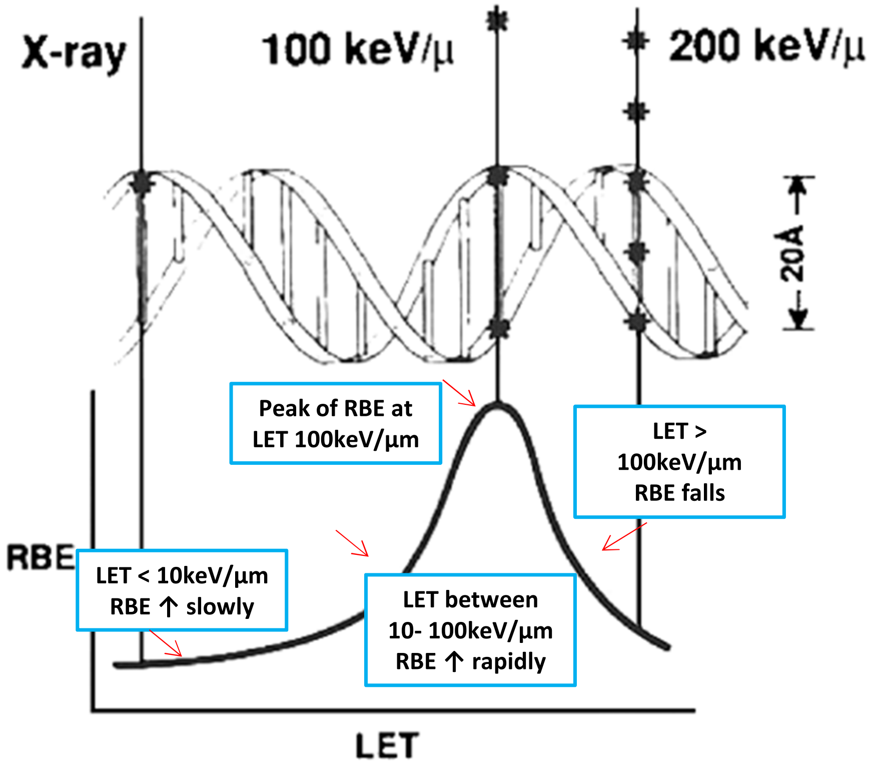
The optimal LET



Modified from Hall, E. & Hei, T. *Oncogene*, 2003

LET of about **100 keV/µm** is optimal in terms of producing a biologic effect.

→ At this density of ionization, **the average separation in ionizing events is equal to the diameter of DNA double helix** which causes significant DSBs.

→ Beyond this value, the energy is wasted as events coincide with each other.

Assumption: DSBs are the basis of most biologic effects (**Cell killing, mutagenesis or oncogenic trasformation)**

What about other biological effects???

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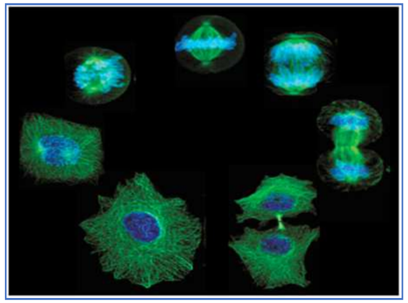
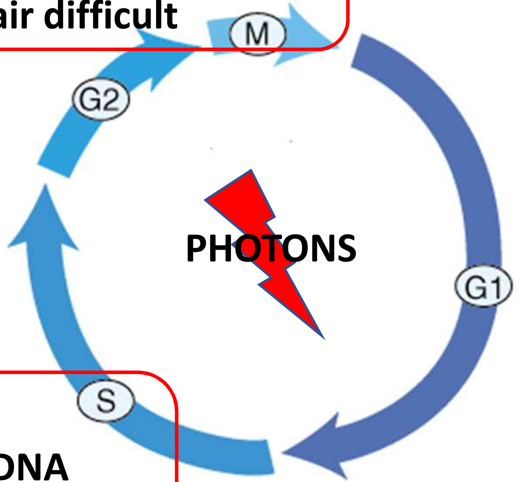
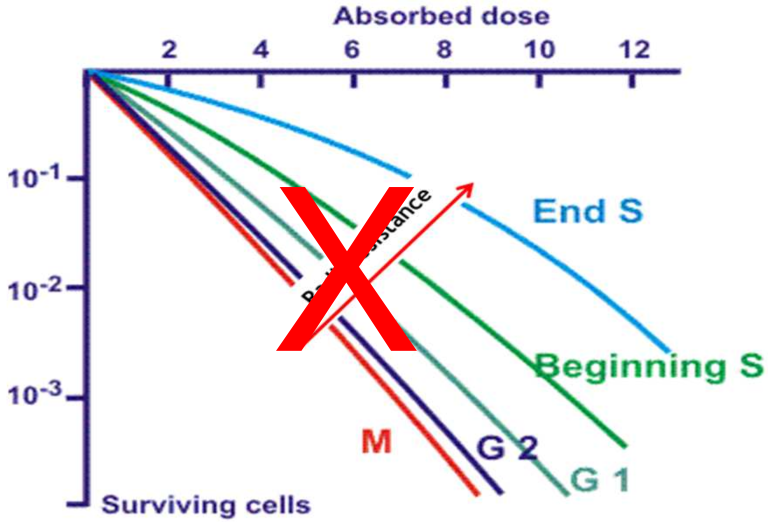
Radiosensitivity cell cycle dependence



Radiosensitive
- chromatin condensation
  DNA repair difficult



Radioresistant
- **Duplicated DNA strands provide template for repair**



High LET radiation: No significant effects on radiosensitivity through the cell cycle when delivering high LET radiation

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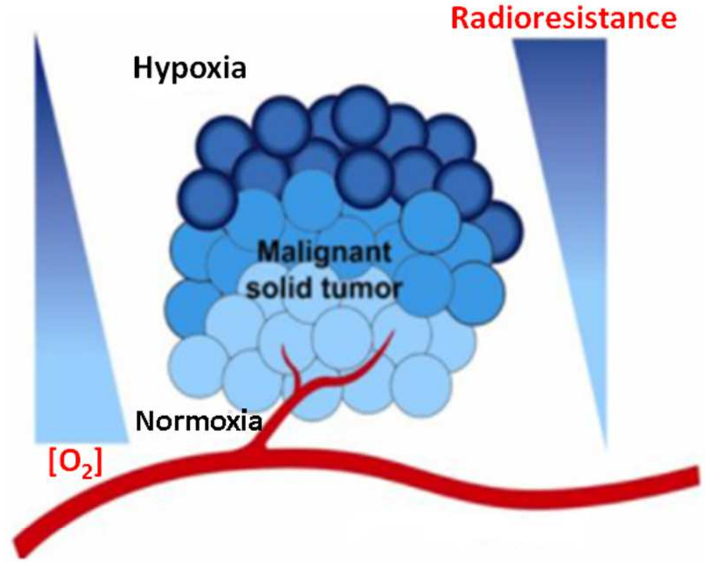
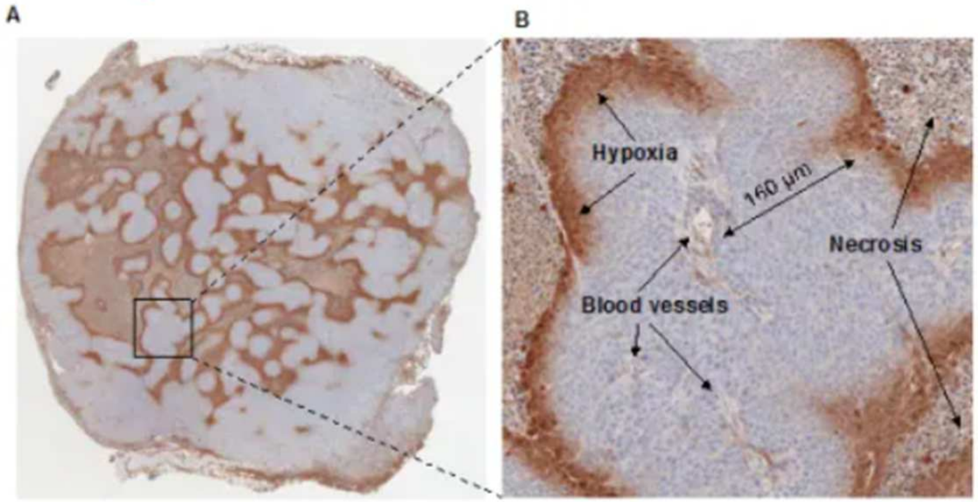
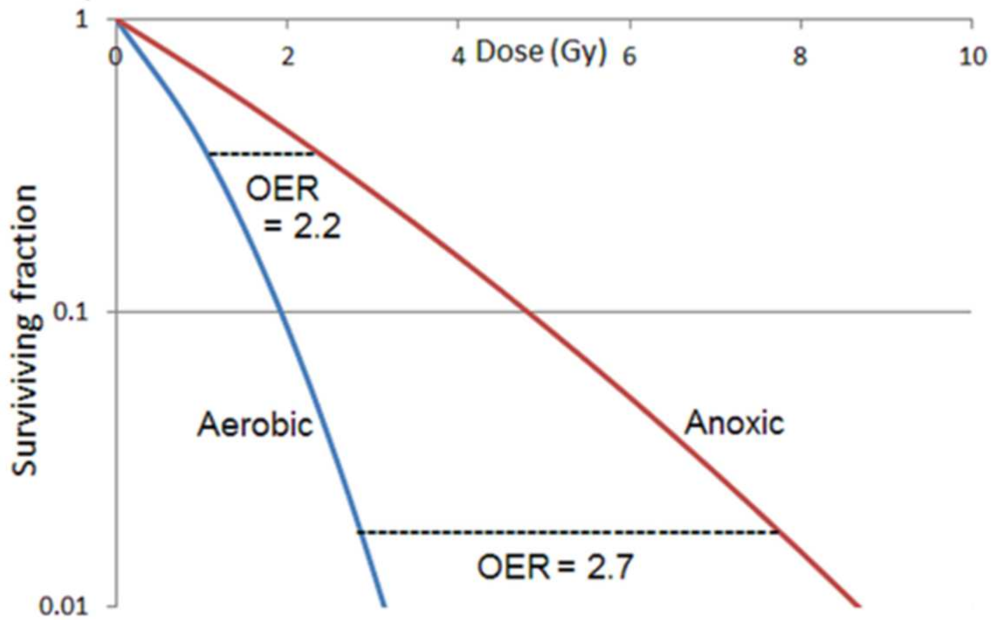
The Oxygen effect





https://www.oncology.ox.ac.uk/research/research-group/tumour-hypoxia



$$OER = \frac{D_{\text{hypoxic}}}{D_{\text{oxic}}}\bigg|_{\text{isoeffect}}$$

<u>Oxygen Enhancement Ratio (OER):</u>

The ratio of HYPOXIC to AEROBIC doses needed to achieve the SAME biological effects

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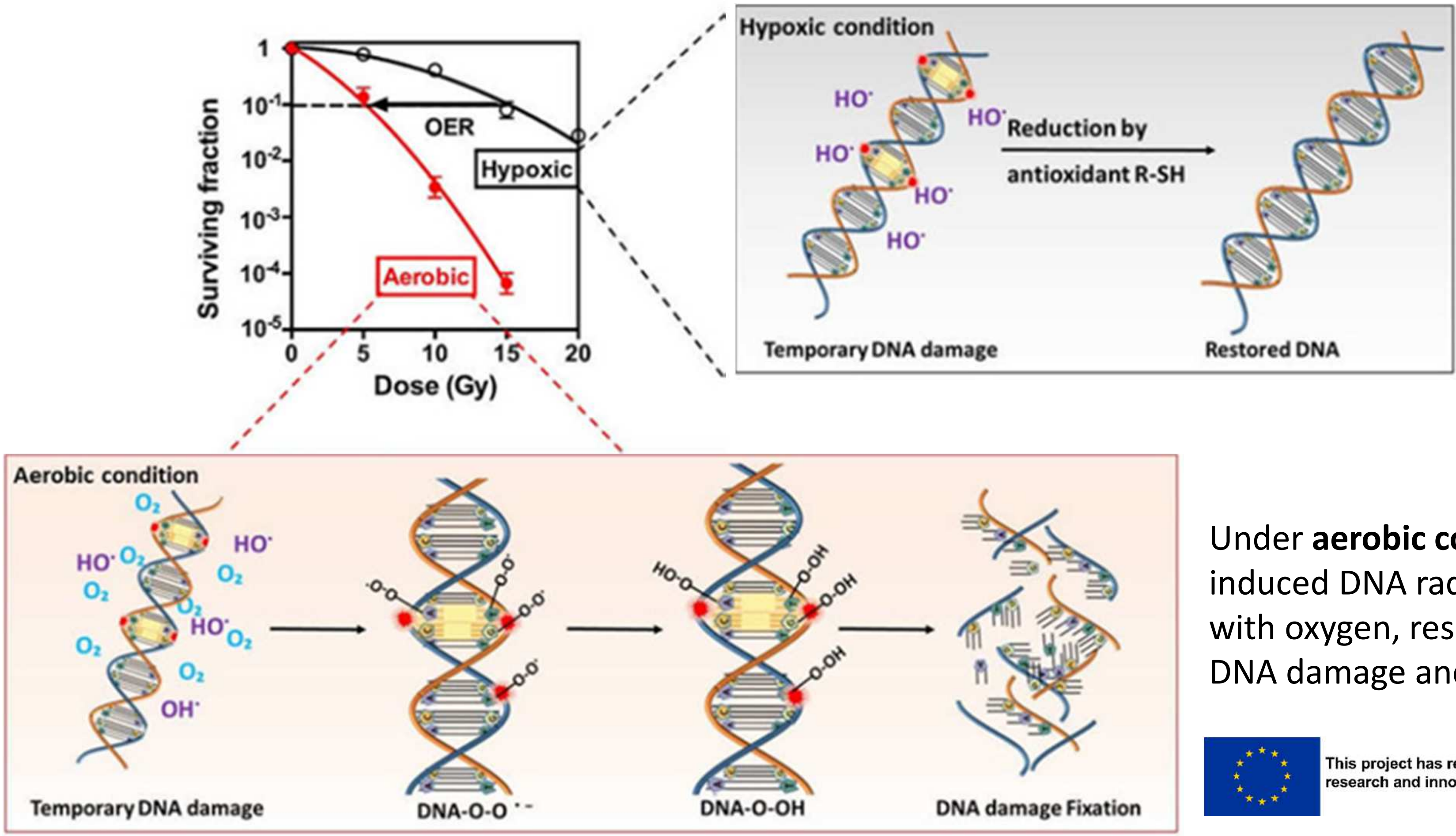
HYPOXIA- INDUCED RADIORESISTANCE:
The oxygen fixation hypothesis



Under **hypoxic conditions**, the lack of oxygen enables the DNA radicals to be reduced to the original form that hampers the generation of strand breaks.

Under **aerobic conditions**, radiation induced DNA radicals are able to react with oxygen, resulting in permanent DNA damage and strand breaks.

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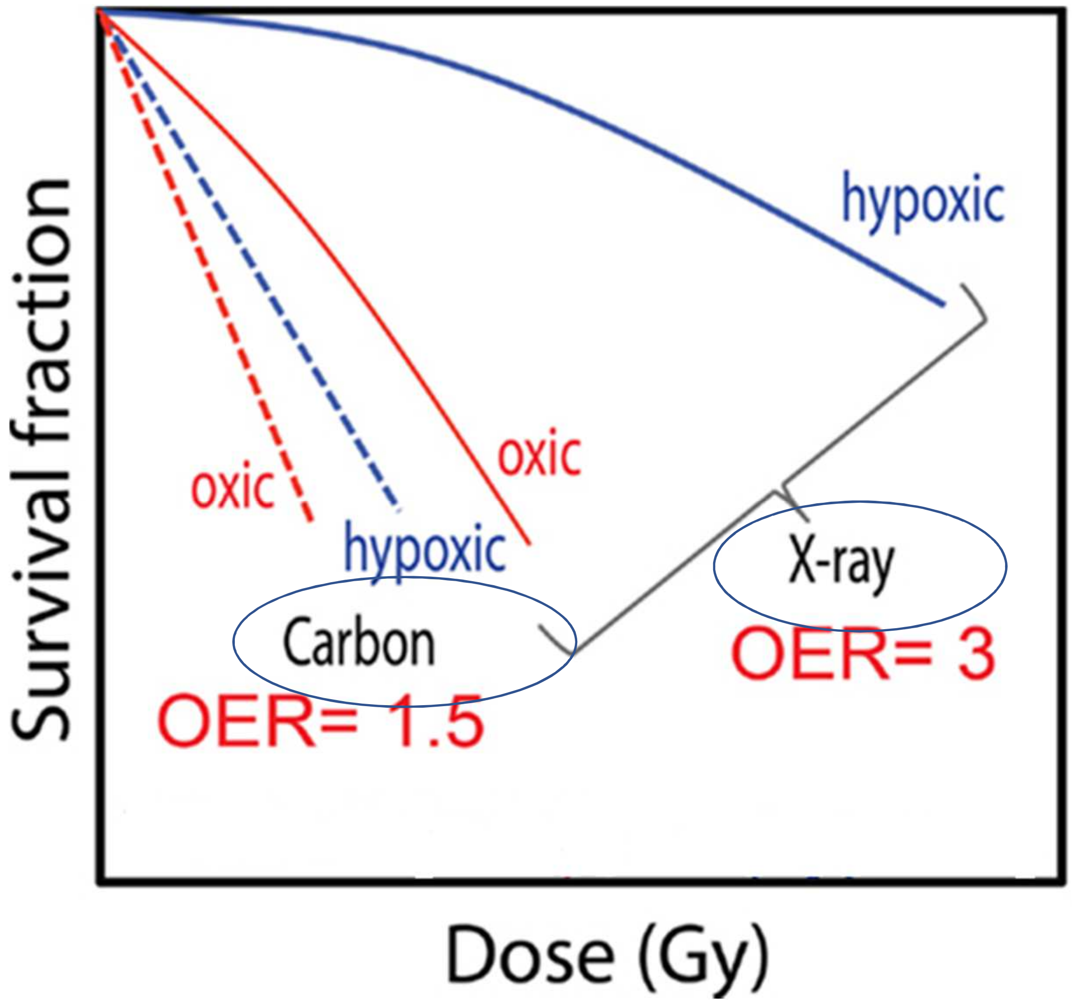
Carbon-ion irradiation is able to reduce hypoxia-induced radioresistance



The oxygen effect is reduced with high-LET carbon-ion irradiation

WHY???

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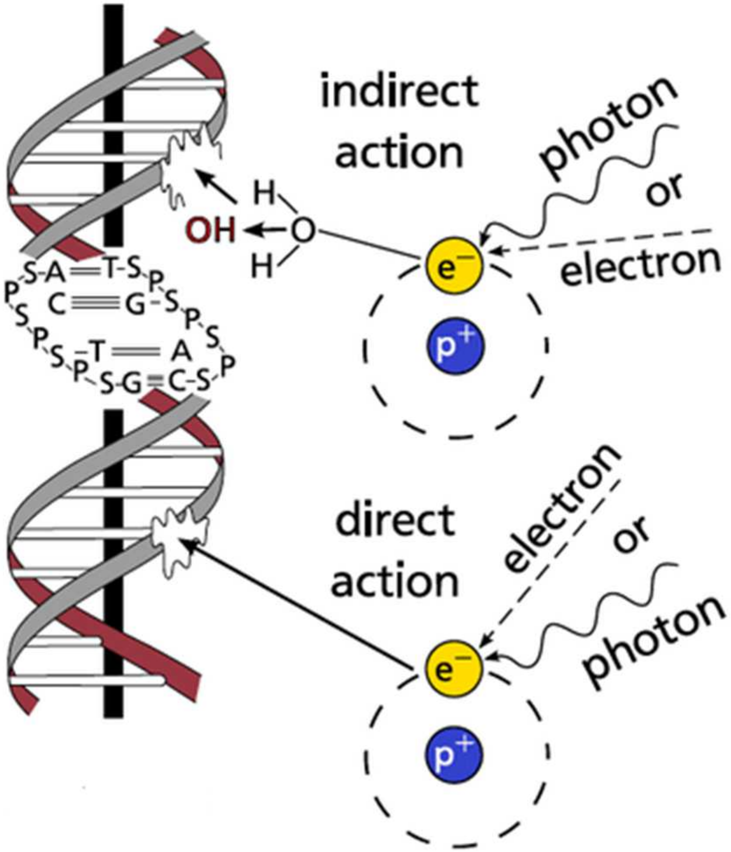
Direct and indirect actions of radiation



Dominant for low LET radiation

Radiation interacts with other molecules in the cells, particularly water, to produce **free radicals** such as hydrogen atoms (H^+), hydroxyl radicals (HO), and superoxide radical anion (O2 −), which in turn induce the damage to the DNA.

Dominant for high LET radiation

Radiation directly interacts with DNA resulting in DNA damage.

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Fate of irradiated cells (DNA-centered)

- **No effect**.
- **Division delay**: The cell is delayed from going through division.
- **Apoptosis**: The cell dies before it can divide or afterwards by fragmentation into smaller bodies, which are taken up by neighbouring cells.
- **Reproductive failure**: The cell dies when attempting the first or subsequent mitosis.
- **Genomic instability**: There is a delayed form of reproductive failure as a result of induced genomic instability.
- **Mutation**: The cell survives but contains a mutation.
- **Transformation:** The cell survives but the mutation leads to a transformed phenotype and possibly carcinogenesis.
- **Bystander effects**: An irradiated cell can send signals to neighbouring unirradiated cells and induce genetic damage in them.
- **Adaptive responses**: The irradiated cell is stimulated to react and become more resistant to subsequent irradiation.

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«Rock» radiation biology for hadrontherapy

Radiation causes damage to all cellular molecules, but DNA damage is most critical (most cellular and molecular components can be replaced)

BUT…

Cellular organelles are important targets of IR and they play a significant role in mediating radiation effects. It has been shown that radiation can damage the endoplasmic reticulum, induce changes in the ribosome, damage the lysosome, affect the biological properties and the signal transduction of the plasma membrane, and affect mitochondrial function AND…..

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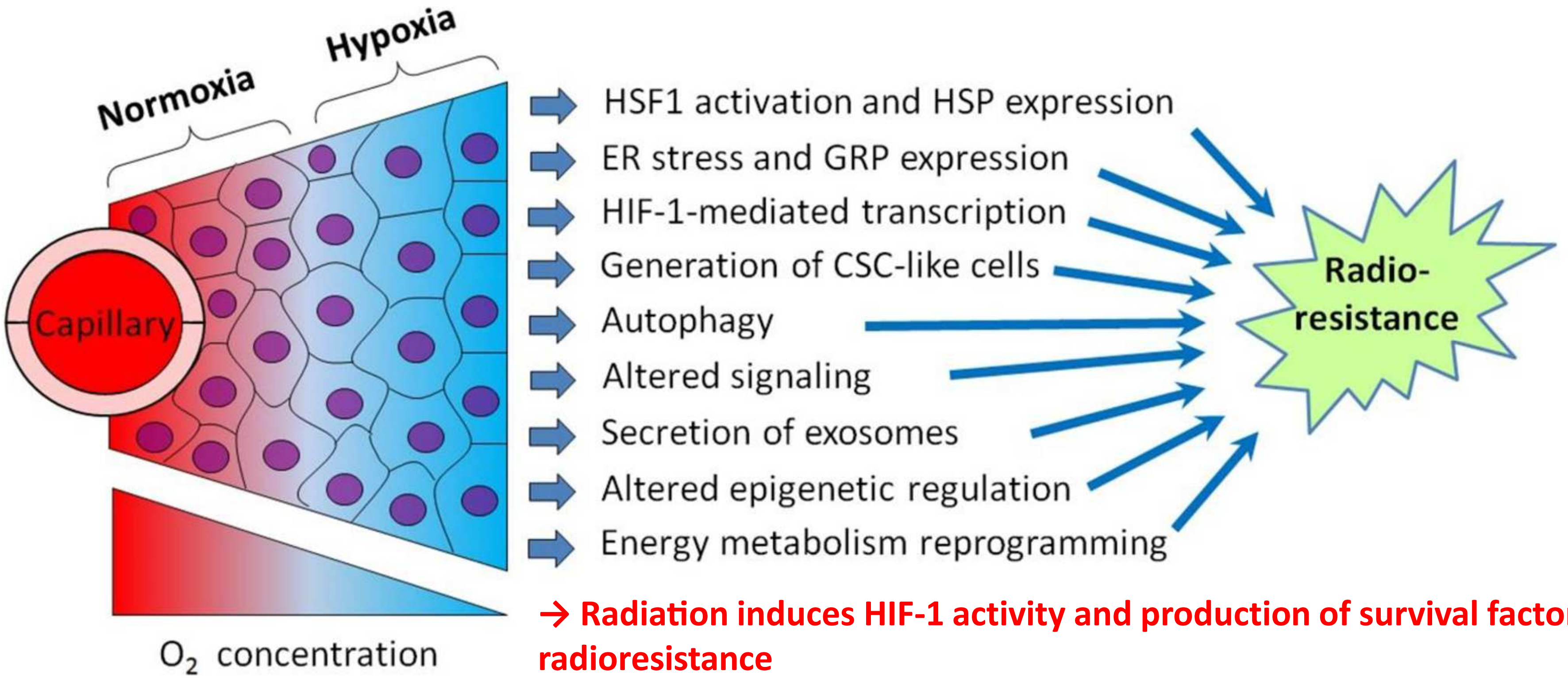
Hypoxia-induced radioresistance: biological oxygen effect

Major hypoxia-induced cancer cell responses promoting the radioresistance of hypoxic tumors:



→ Radiation induces HIF-1 activity and production of survival factors and consequently radioresistance

HSF1—heat shock factor 1, HSP—heat shock protein, ER—endoplasmic reticulum, GRP—glucose-regulated protein, HIF-1—hypoxia-inducible factor-1 and CSC—cancer stem cell

Kabakov A.E and Yakimova AO, 2021

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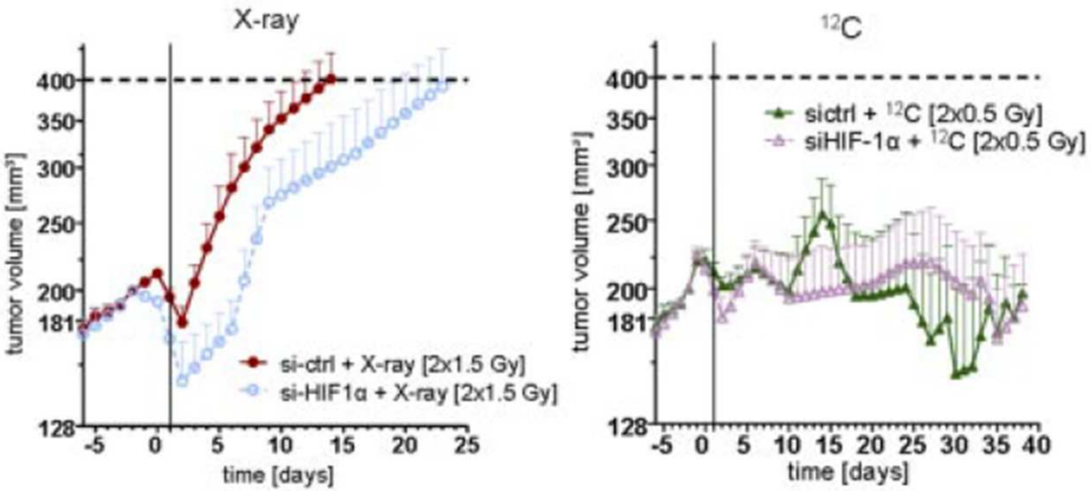
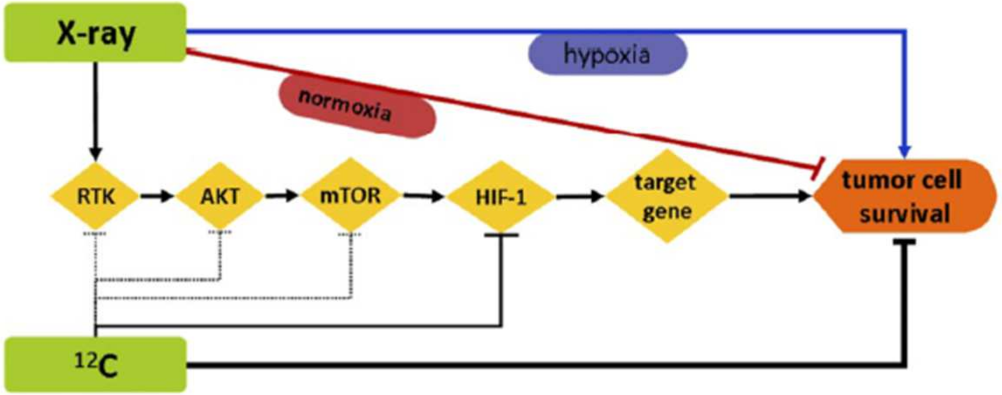
The FASEB Journal • Research Communication

Carbon ion radiotherapy of human lung cancer attenuates HIF-1 signaling and acts with considerably enhanced therapeutic efficiency

Florentine S. B. Subtil,*[,1] Jochen Wilhelm,[‖] Verena Bill,* Niklas Westholt,* Susann Rudolph,* Julia Fischer,* Sebastian Scheel,* Ulrike Seay,[‖] Claudia Fournier,[¶] Gisela Taucher-Scholz,[¶] Michael Scholz,[¶] Werner Seeger,[‖] Rita Engenhart-Cabillic,*[,§] Frank Rose,* Jochen Dahm-Daphi,[†] and Jörg Hänze[‡]



A549 tumor volume curves after photon and carbon ion irradiation



Carbon ion and photon irradiation differ with respect to mTOR-dependent HIF-1 signaling, with opposing consequences for tumor cell survival

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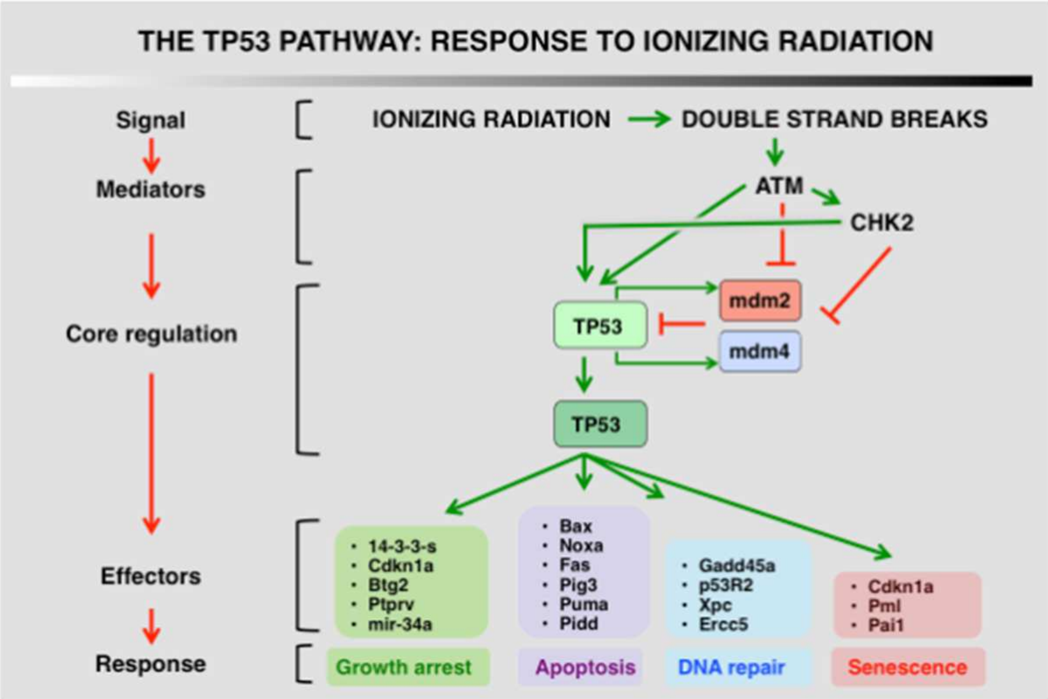
P53 mutations and radioresistance



https://p53.fr/tp53-information/tp53-knowledge-center/26-knowledge-center/28-p53-pathways#the-tp53-pathway-response-to-ionizing-radiation

Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 5742–5746, June 1993
Genetics

p53 mutations increase resistance to ionizing radiation

(γ radiation/DNA damage/transgenic mice/carcinogenesis)

JONATHAN M. LEE* AND ALAN BERNSTEIN*†‡

*Division of Molecular and Developmental Biology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, ON, Canada M5G 1X5; and †Department of Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada M5S 1A8

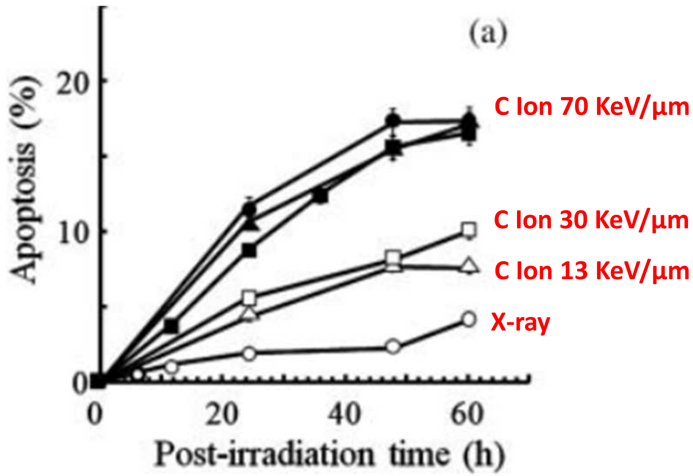
TP53 mutations increase radioresistance in rhabdomyosarcoma and Ewing sarcoma

Dana L. Casey[1,2,3], Kenneth L. Pitter[1], Leonard H. Wexler[4], Emily K. Slotkin[4], Gaorav P. Gupta[2,3] and Suzanne L. Wolden[1]

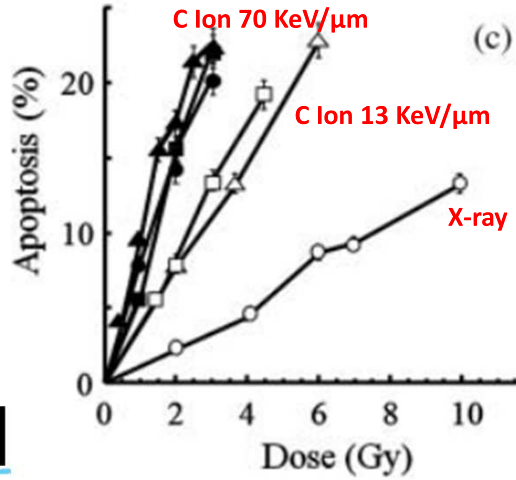
British Journal of Cancer (2021) 125:576–581; https://doi.org/10.1038/s41416-021-01438-2

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101008548

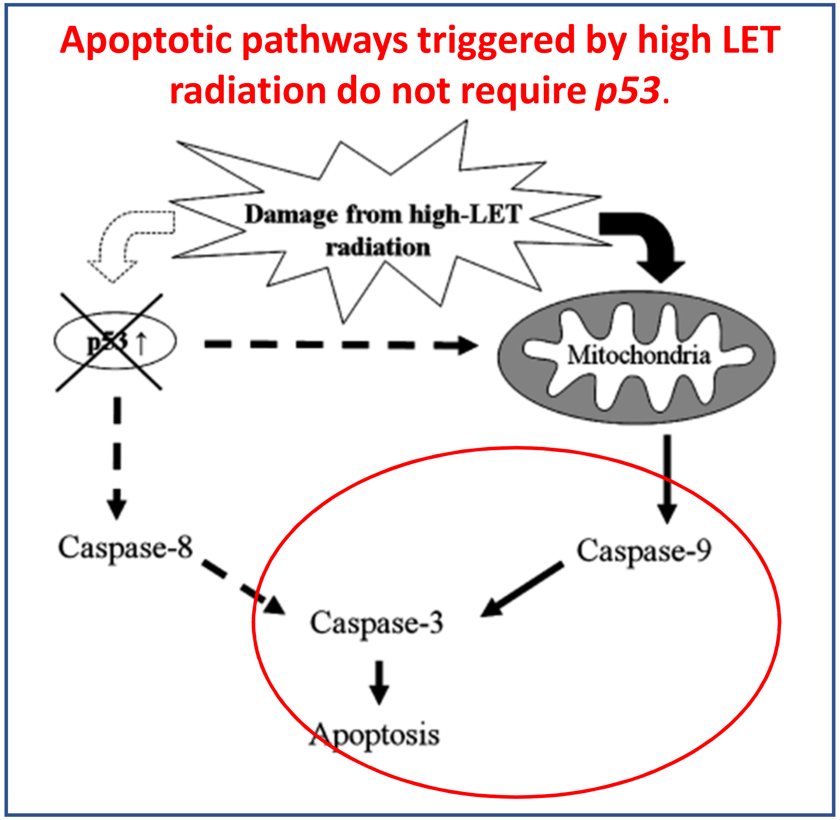
P53-independent apoptosis induced by high LET radiation



Time course of apoptosis induced by X-ray and heavy-ion beam irradiation with 2 Gy in p53 mutated cells



The induction of apoptosis as a function of dose in p53 mutated cells.



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Yamakawa N et al Cancer Sci, 2008

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Migration/invasion

There is clear evidence that ionizing radiation can differently modulate migration and invasiveness of cancer cells depending on the cell lines, the doses and the radiation types investigated

Migration and invasiveness of human tumor cell lines after irradiation.

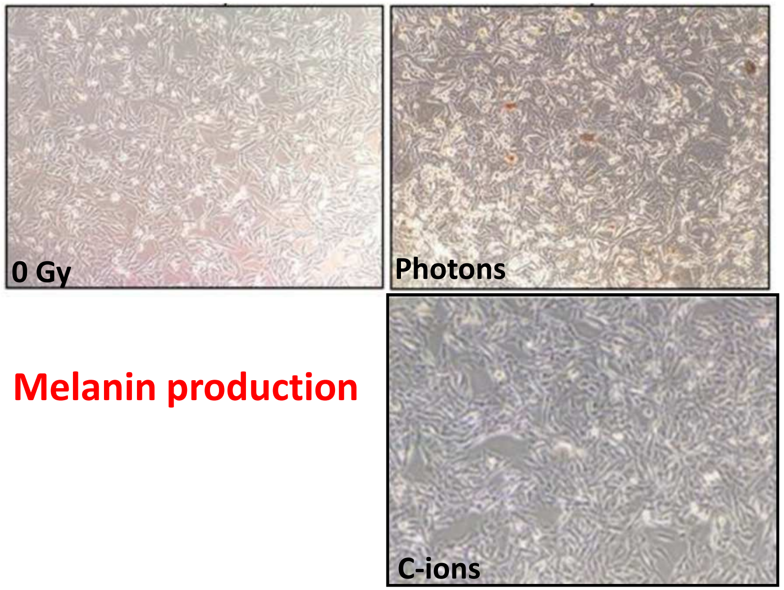
Organ	Cell line	Radiation Dose (Gy) (LET)	Migration	Invasion	Key molecules	Reference
CNS	U87	γ-ray: 2, 10	+ at 2 Gy	N.D.	αvβ3, αvβ5	[55]
		C-ion: 0.5, 3 (91.5 ± 1.5 keV/μm)	– at both doses	N.D.		
	U87	X-ray: 1, 3, 10	N.C. at all doses	N.D.	β3 and β1 integrin	[56]
		C-ion: 1, 3, 10	– at 3, 10 Gy	N.D.	(partial correlation)	
	U87 EGFR++	X-ray: 2, 6	+ at 2 Gy, – at 6 Gy	N.D.	EGFR/AKT/ERK1/2	[58]
		C-ion: 2, 6 (100 keV/mm)[a]	– at both doses	N.D.		
	LN229 EGFR++	X-ray: 2, 6	– at both doses	N.D.	EGFR/AKT/ERK1/2	[58]
		C-ion: 2, 6 (100 keV/μm)[a]	– at both doses	N.D.		
	SF126	X-ray: 4	N.D.	+	–	Unpublished data
		C-ion: 2 (80 keV/μm)[b]	N.D.	+	NOS/PI3K/AKT2/RHOA	[93]
Colon	HCT116	X-ray: 1, 3, 10	– at 10 Gy	N.D.	β1 integrin (partial	[56]
		C-ion: 1, 3, 10	– at all doses	N.D.	correlation)	
	HCT116 p21wt	X-ray: 1, 3, 10	– at all doses	N.D.	p21 was not	[63]
		C-ion: 1, 3, 10	– at all doses	N.D.	affected	
	HCT116 p21-/-	X-ray: 1, 3, 10	– at all doses	N.D.		
		C-ion: 1, 3, 10	– at all doses	N.D.		
Lung	A549	X-ray: 0.5, 2, 10	– at 10 Gy	– at 10 Gy	PI3K/AKT	[67]
		C-ion: 0.25, 1, 5 (50 keV/μm)[c]	– at 1, 5 Gy	– at 1, 5 Gy		
	A549	X-ray: 0.5, 2, 10	– at 2, 10 Gy	– at 10 Gy	ANLN	[68]
		C-ion: 0.25, 1, 5	– at all doses	– at 1, 5 Gy		
	A549	X-ray: 2, 8	+ at both doses	N.D.	RHO	[69]
		C-ion: 2, 8(108 keV/μm)[b]	+ at both doses	N.D.		
	EBC-1	X-ray: 0.5, 2, 8	N.C. at all doses	N.C. at all doses	N.D.	[68]
		C-io: 0.25, 1, 4	– at 4 Gy	– at 1, 4 Gy		
Pancreas	MIAPaCa-2	X-ray: 2, 4, 8	+ at 2 Gy, - at 8 Gy	+ at 2, 4 Gy	RHOA/RAC1,	[15]
		C-ion: 2 (80 keV/μm)[b]	–	–	MMP-2	[16]
		C-ion: 0.5, 1, 2, 4 (80 keV/mm)[b]	– at 1, 2, 4 Gy	– at 1, 2, 4 Gy		[102]
	AsPC-1	C-ion: 2 (80 keV/μm)[b]	–	N.C.	–	[16]
	BxPC-3	C-ion: 2 (80 keV/μm)[b]	–	N.C.	–	[16]
	Panc-1	X-ray: 2, 4, 8	N.C. at all doses	+ at 2, 4 Gy	RHOA/RAC1, uPA/plasmin	[16]
		C-ion: 0.5, 1, 2, 4(80 keV/μm)[b]	– at 4 Gy	+ at 1, 2, 4 Gy	NOS/PI3K/AKT2/RHOA/RAC1 uPA/plasmin	[16,93]
Sarcoma	HT1080	X-ray: 0.5, 2, 8	+ at 0.5 Gy	+ at 0.5, 2 Gy, - at 8 Gy	aVb3	[74]
		C-ion: 0.2, 1, 4	– at all doses	– at all doses	MMP-2	
		Proton: 0.5, 2, 8	– at all doses	– at all doses	MMP-2	

Fujita M et al, 2015

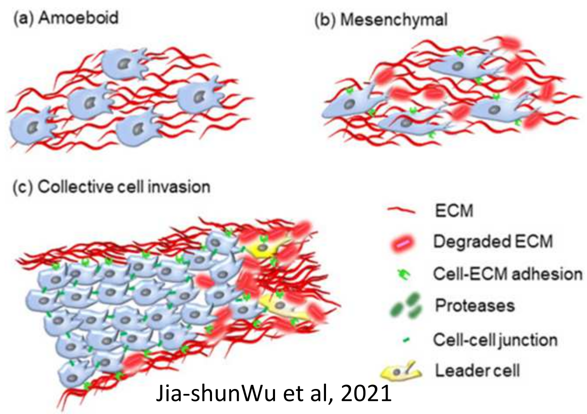
There are not many reports studying the effects of particle radiation-altered cancer cell motility, the majority of cell lines, which has been examined, showed reduced migration and/or invasiveness upon particle irradiation

High and low LET differently differentiate tumour cell?

Dendrites formation and elongation



Melanin production



Jia-shunWu et al, 2021

Different patterns of tumour cell migration

Morphological Analysis of Amoeboid–Mesenchymal Transition Plasticity After Low and High LET Radiation on Migrating and Invading Pancreatic Cancer Cells

ANGELICA FACOETTI, CARMELA DI GIOIA, FRANCESCA PASI, RICCARDO DI LIBERTO, FRANCO CORBELLA, ROSANNA NANO, MARIO CIOCCA, FRANCESCA VALVO and ROBERTO ORECCHIA

Anticancer Research August 2018, 38 (8) 4585-4591; DOI: https://doi.org/10.21873/anticanres.12763

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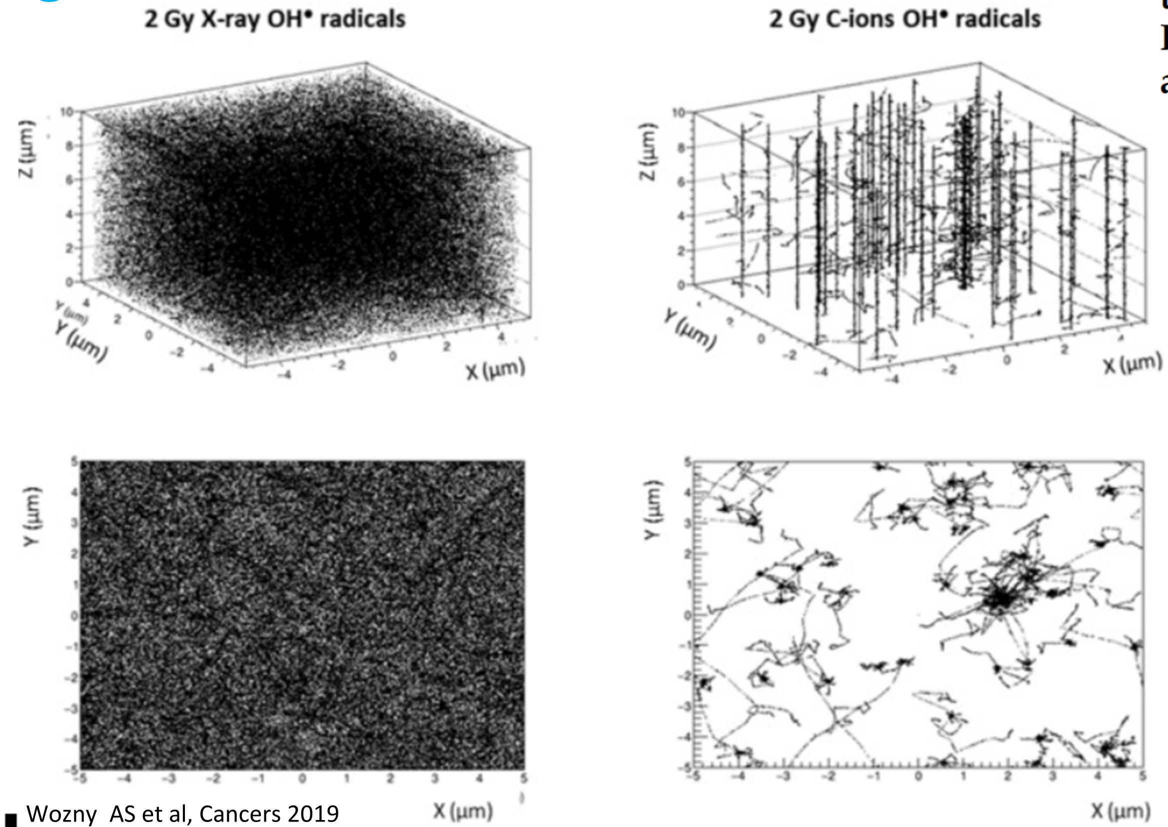
ROS distribution and the signaling pathways involved in migration/invasion



Wozny AS et al, Cancers 2019

HO• radical distribution in response to X-rays and to a mixed radiation field reproducing the NIRS irradiation in C-ions SOBP

cancers

MDPI

Article

ROS Production and Distribution: A New Paradigm to Explain the Differential Effects of X-ray and Carbon Ion Irradiation on Cancer Stem Cell Migration and Invasion

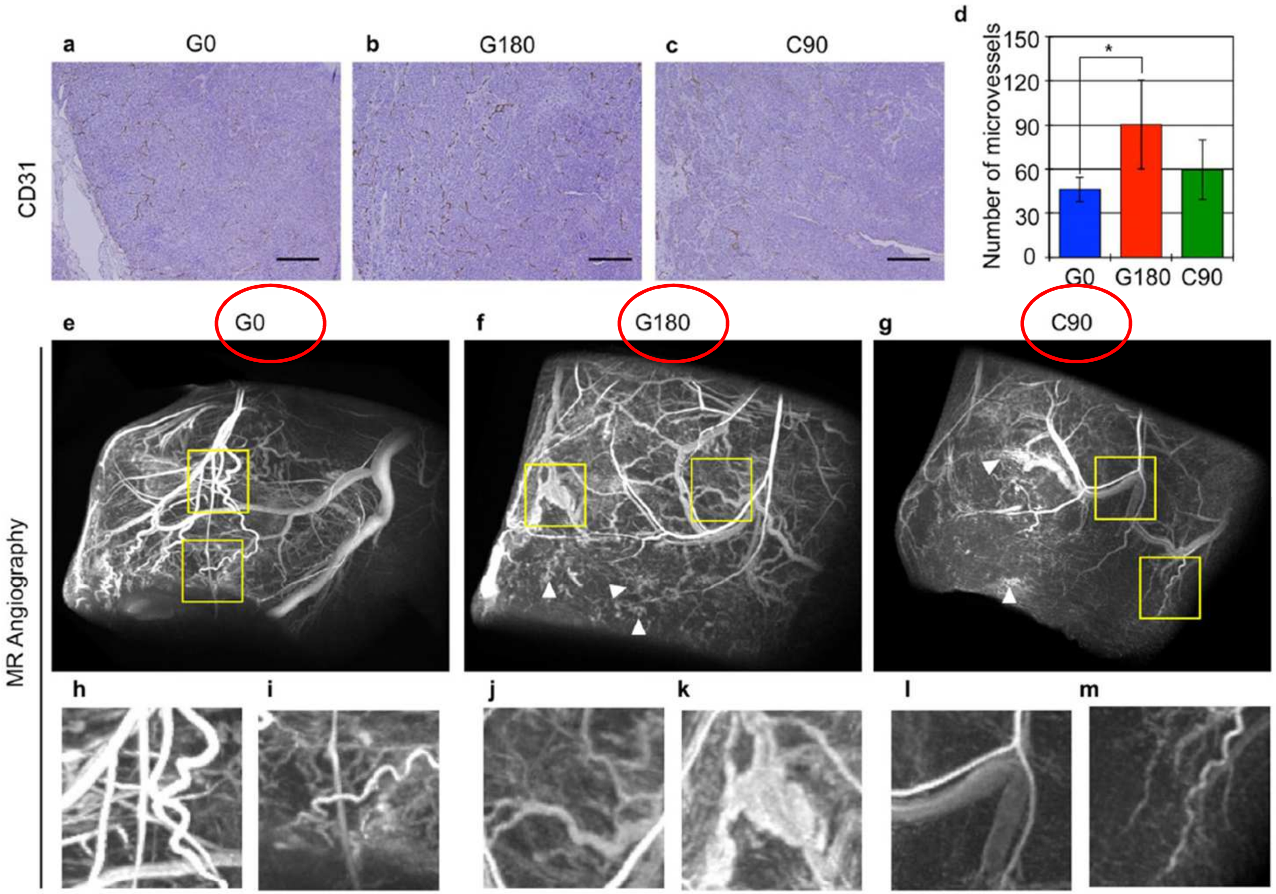
The specific ROS distribution observed in response to C-ions can induce complex DNA lesions and cell death, however it may preserve the plasma membrane and intracellular structures of cells outside the ion tracks and does not allow the achievement of the threshold of ROS that is necessary to activate the signaling pathways involved in migration/invasion.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101008548

SCIENTIFIC REPORTS

Repeated photon and C-ion irradiations *in vivo* have different impact on alteration of tumor characteristics

Katsutoshi Sato[1,2], Nobuhiro Nitta[3], Ichio Aoki[3], Takashi Imai[4] & Takashi Shimokawa[1]



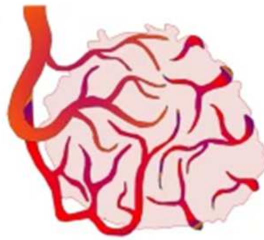


Figure 5. Evaluation of tumor microvessels using immunohistochemical staining and magnetic resonance angiography. (**a–c**) show the tissue section immunostaining with anti-CD31 antibody. The scale bar shows 250 µm. (**d**) shows the number of CD31 positive microvessels in each tumor. The values indicated the mean ± standard deviation. The asterisk means statistical significant compared with the value of G0 tumor. (**e–m**) Shows 3D MR micro-angiography of the indicated tumor. (**h,i**), (**j,k**) and (**l,m**) were indicated that the magnified images of the area enclosing yellow square in (**e**), (**f**) and (**g**), respectively.

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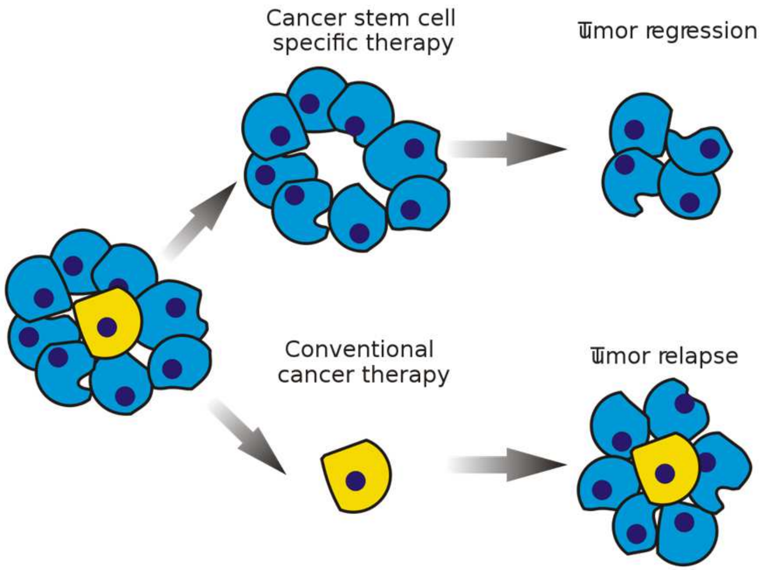
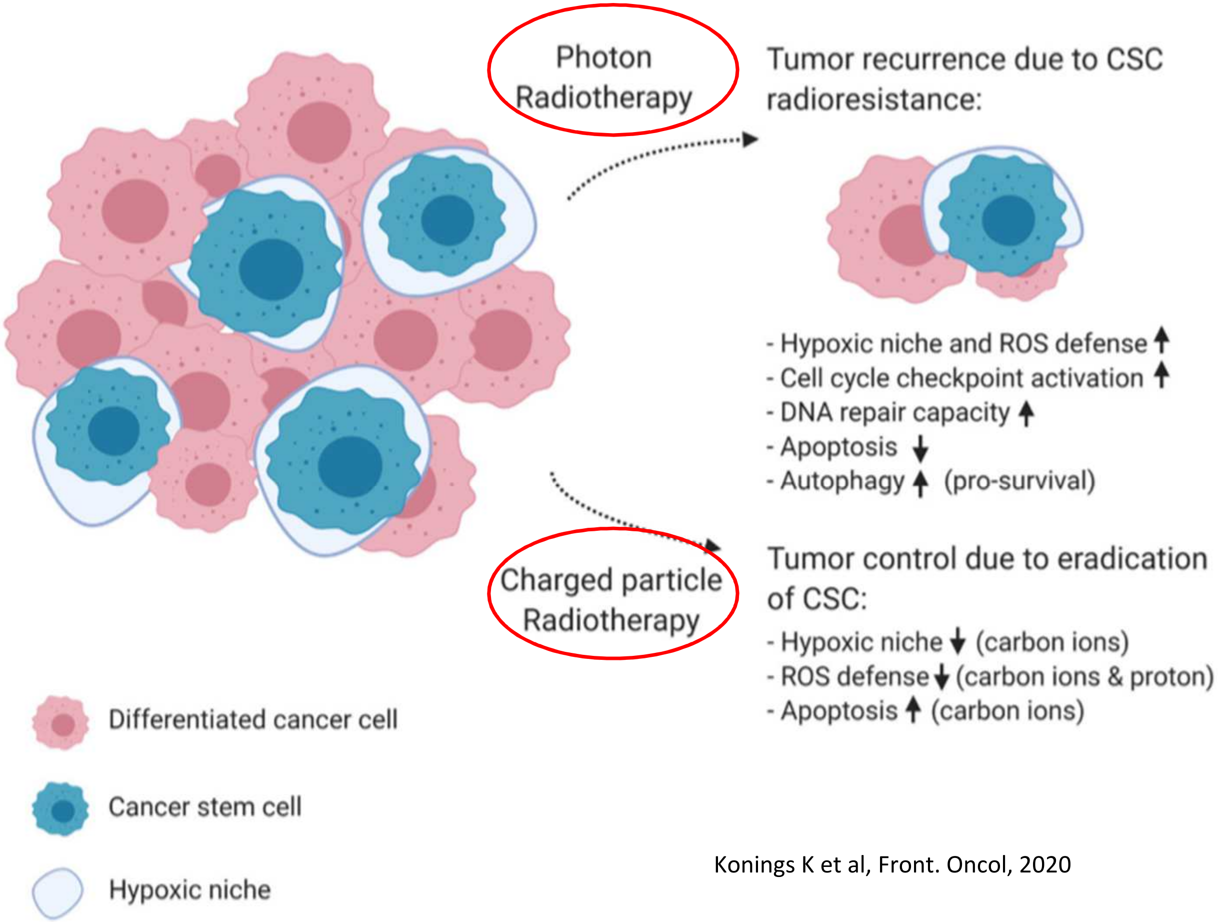
under grant agreement No 101008548

Sat K et al, 2018

TUMOR RADIORESISTANCE: Cancer Stem Cells





Konings K et al, Front. Oncol, 2020

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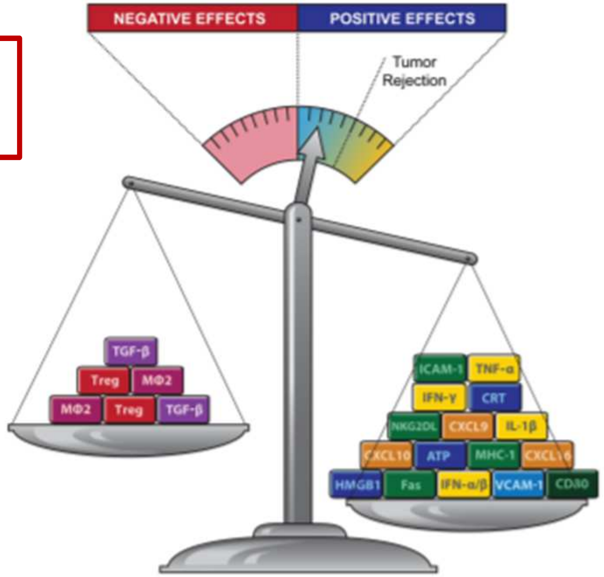
High LET radiation: from immunosuppressive to pro-immunogenic signals - Shifting the balance

RT activates immunosuppressive factors



Radiation promotes the priming and effector phases of the antitumor immune response

*"**positive effects of RT often predominate over negative** ones but are **insufficient** to shift the balance of the immunosuppressive tumor microenvironment to achieve tumor rejection **in the absence of targeted immunotherapy**"*

Radiobiology of HIGH LET radiation may help to shift this balance

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Formenti SC, Demaria S. J Natl Cancer Inst. 2013; Durante M et al Int J Radiat Oncol Biol Phys. 2016; Durante M et al Int J Radiat Oncol Biol Phys. 2000

Immunogenic properties of Carbon ions

- ✓ Although basic science studies are still very limited, recent several *in vitro*, *ex vivo* and *in vivo* studies suggest that charged particles may be more immunogenic than photons.
- ✓ Carbon ion radiation increased the levels of high mobility group box 1 (HMGB1) in the culture supernatants of different human cancer cell lines (Yoshimoto Y et al, 2015).
- ✓ Preliminary *in vitro* studies showed an increased release of immune-stimulating cytokines after heavy ion exposure (*Durante M & Formenti S. 2019)*.
- ✓ Carbon ions, may distinctly affect cell death pathways, leading to increased immunogenicity.
- ✓ Classic protracted regimens of fractionated radiation therapy induce some degree of lasting lymphopenia, by exposure of circulating blood during treatment and inclusion of active hematopoietic organs within relevant dose volumes. The more favorable integral dose of particle therapy likely reduces this effect.

Carbon ions may lead to a broader immunogenic response

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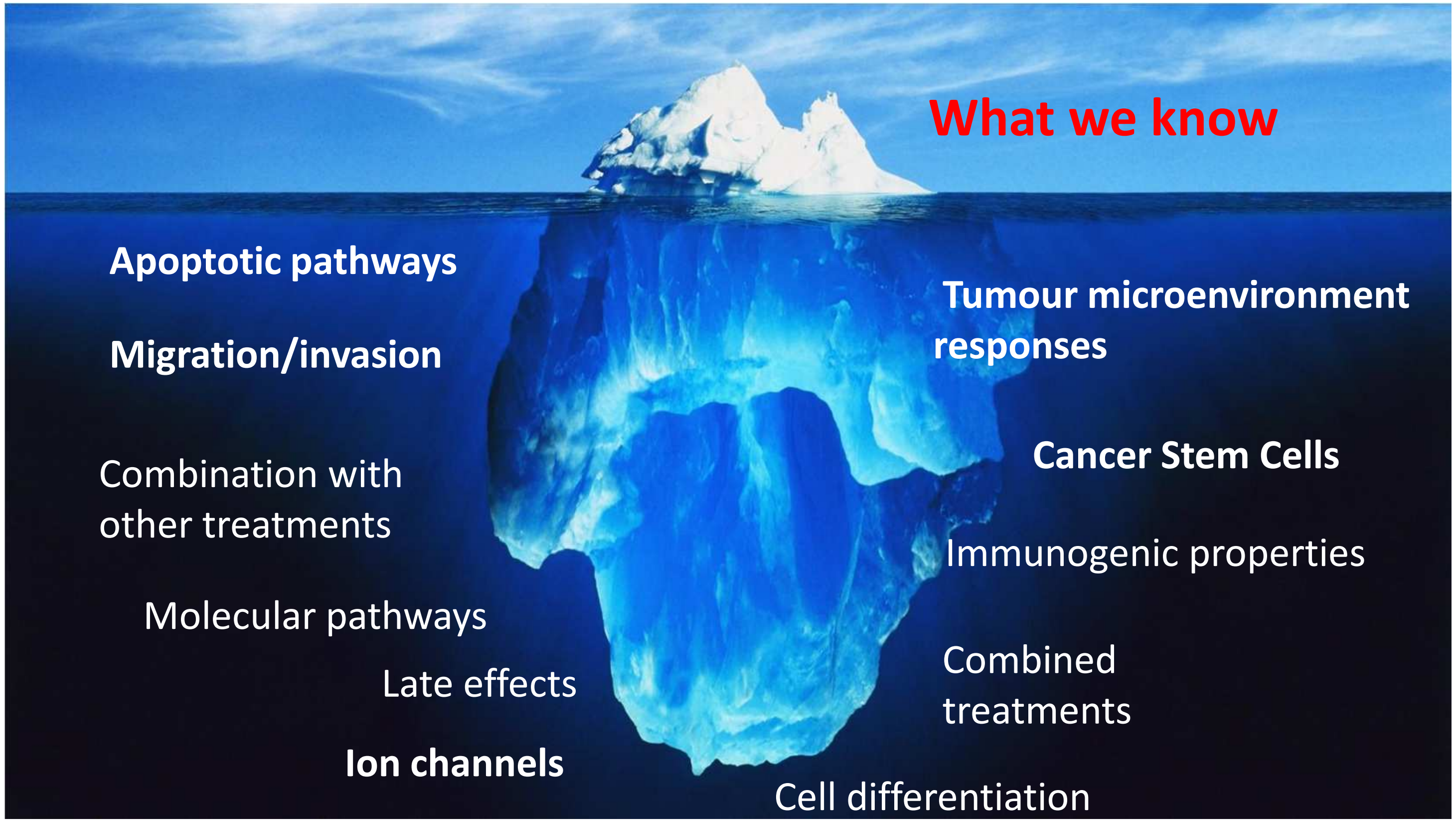
Particle radiobiology



Radiobiology of densely ionizing radiation is so markedly different than X-rays that charged particles should be regarded in radiotherapy in much the same way as a <u>different drug</u> is treated in medical oncology

Tinganelli W. & Durante M. Cancers 2020

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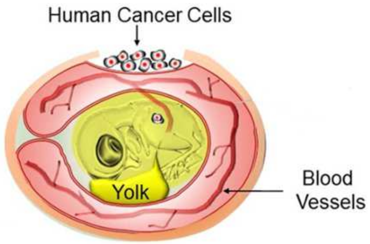
Recomandations:

- Move to more *in vivo*-like conditions…3D models, microenvironment, co-coltures…

- *In vivo* «alternative» models, not necessary small rodents: zebra fish, in ovo…



- Interact with other specialities/professionels

- Do not follow the fashions of the moment

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Thanks!

"*This material was prepared and presented within the HITRIplus* ***Specialised Course on Heavy Ion Therapy Research,*** *and it is intended for personal educational purposes to help students; people interested in using any of the material for any other purposes (such as other lectures, courses etc.) are requested to please contact the authors (angelica.facoetti@cnao.it).*

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