Effect of low dose fracionated radiation on cytotoxicity of cisplatin and paclitaxel in cervix cancer cell lines

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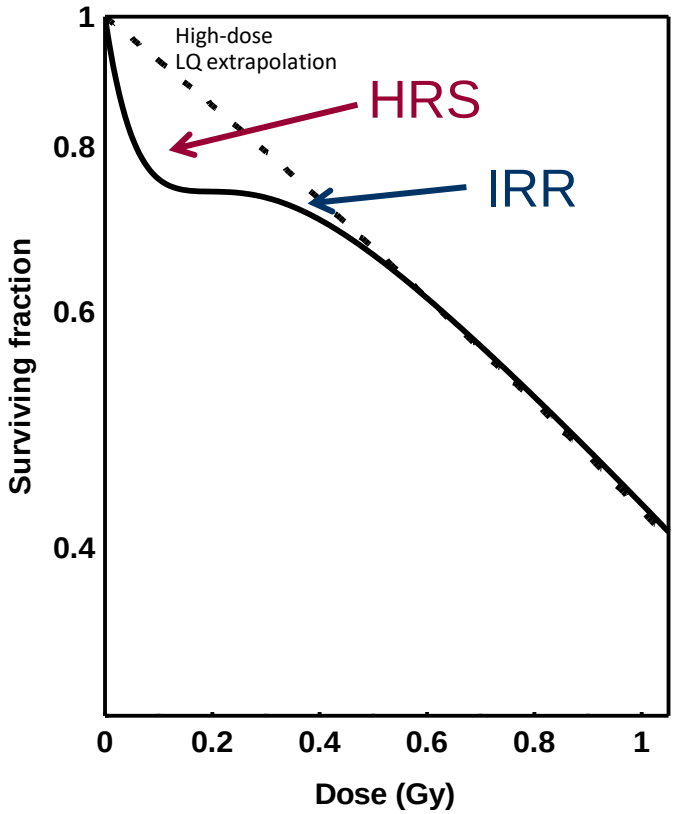
Low-dose hyper-radiosensitivity (HRS)



HRS – increased sensitivity (cell killing) to radiation doses below ~ 0.3 Gy

IRR- increased radioresistance to radiation doses above ~ 0.3 Gy

Marples and Joiner. Radiat Res (1993) 133, 41-45

Rationale for clinical exploiting of Low Dose Fractionated Radiation Therapy (LDFRT) combined with chemotherapy

LDFRT potentiates the cytotoxic effects of chemotherapy

taxanes (in head and neck tumor cells, colorectal tumor cells, gastric cancer cells)

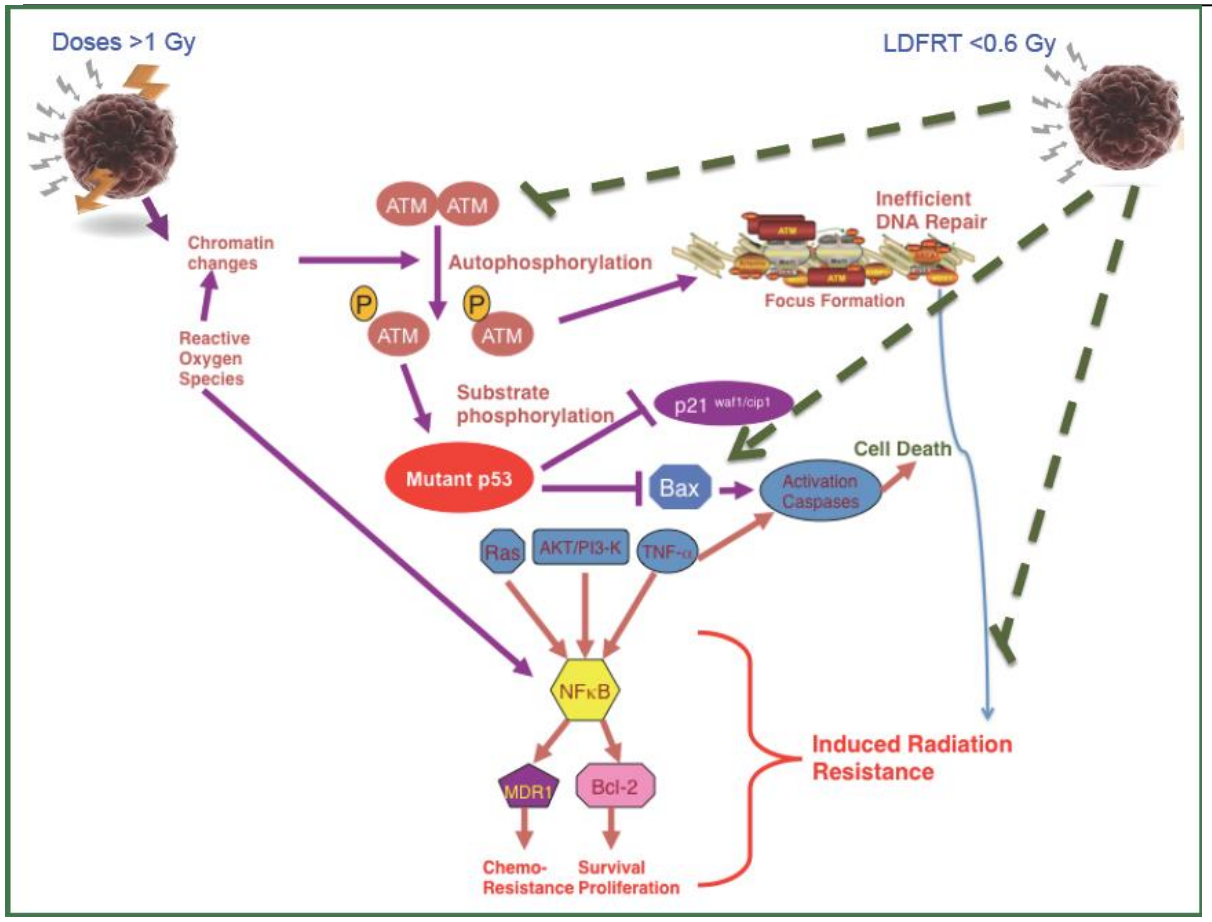
(Chendil et al. 2000, Dey et al. 2003, Spring et al. 2004, Balcer-Kubiczek et al. 2008),

cisplatin (in human lung cancer cells) *Modesitt et al. 2005, Gupta et al. 2011*.

LDFRT does not induce MDR1 protein responsible for multidrug-resistance

Spring et al. Cell Cycle (2004) 3, 479-485, *Shareef et al. Mol Cancer Res (2008) 6, 89-98*

Molecular mechanisms underlying the process of chemopotentiation by LDFRT

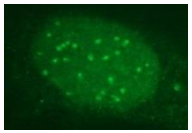


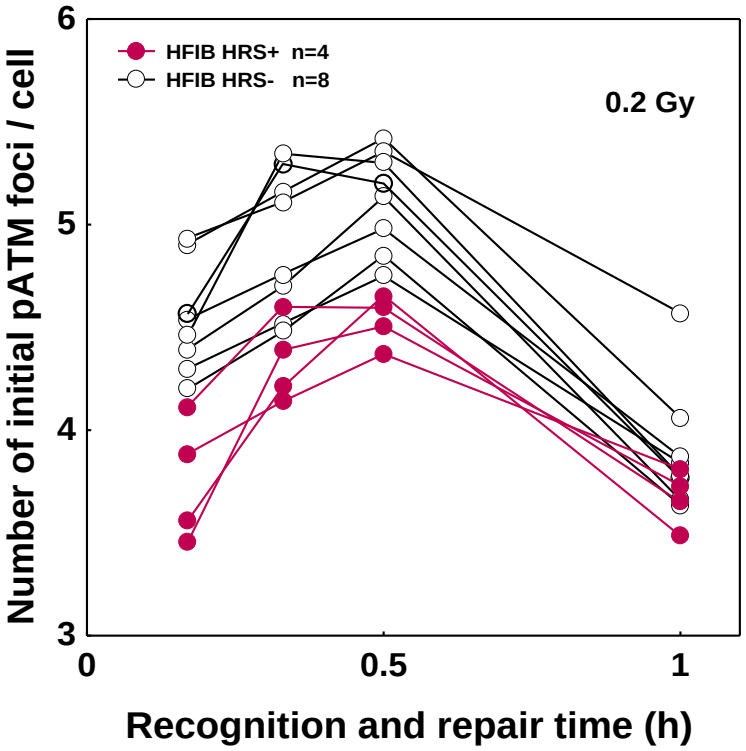
Prasanna et al. J. Thorac. Dis. (2014) 6, 287-302

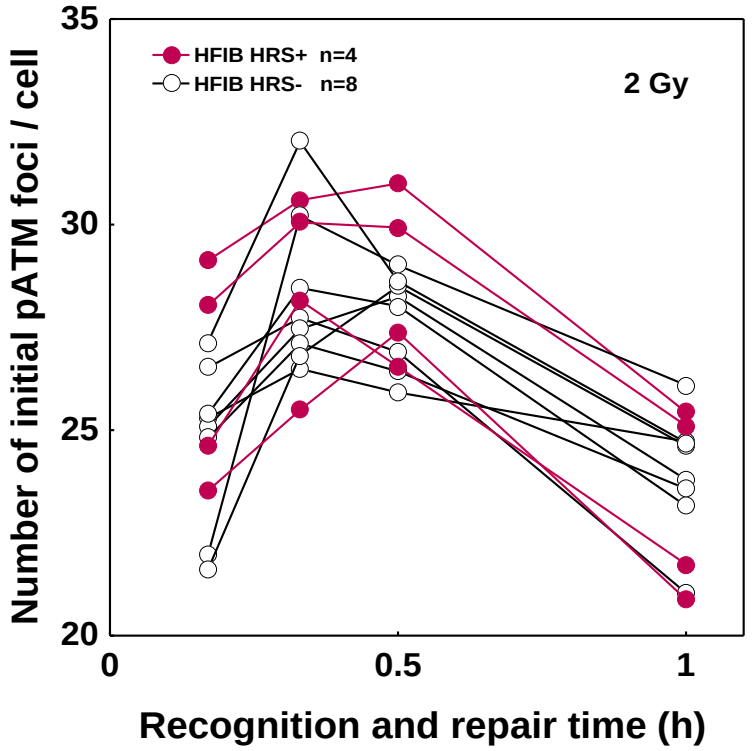
- Lack of activation of ATM kinase
- No DNA repair
- Lack of increase in NFkB activity as well as in Bcl2 and MDR1 proteins
- Activation Bax protein and apoptotic pathway

Kinetics of pATM foci appearance and disappearance after 10 minutes up to 1 hour after irradiation with 0.2 Gy and 2 Gy for HRS-positive and HRS-negative fibroblasts

pATM assay







Słonina et al. Int. J. Radiat. Oncol. Biol. Phys. (2018) 100, 756-766

Phase II clinical trials combining LDFRT with chemotherapy in solid tumors

- Phase II trial: ChT (paklitaxel+carboplatin) + LD-FRT (0.8 Gy/fr) in 40 patients with locally advanced head and neck cancer *(Arnold et al. 2004, Gleason et al. 2013, Silver et al. 2015, Arnold et al. 2016)*.
- Phase II trial: ChT (pemetrexed) + LD-FRT (0.4 Gy/fr) in 19 patients with recurrent non-small-cell lung cancer *(Mantini et al. 2012).*
- Phase II trial: Cht (doxorubicyne+docetaxel) + LD-FRT (0.4 Gy/fr) in 21 patients with stage IIA-IIIA breast cancer *(Nardone et al. 2012, 2014, Buffi et al. 2012).*
- Phase II trial: ChT (paclitaxel + carboplatin) + LD-FRT (0.8 Gy/fr) in 24 patients with locally advanced carcinoma of the uterine cervix *(Das et al. 2015)*.
- Phase II trial: ChT (FOLFIRI-bewacizumab) + LD-FRT (0.2 Gy/fr) in 18 patients with metastatic colorectal cancer *(Morganti et al. 2016)*.
- Phase II trial: ChT (cetuximab+docetaxel) + LD-FRT (0.5 Gy/fr) in patients with recurrent unresectable locally advanced head and neck carcinoma *(Patel et al. 2016) ClinicalTrials.gov NCT01794845*
- Phase II trial: ChT (temozolomide) + LD-FRT in patients with recurrent anaplastic astrocytoma or glioblastoma multiforme *(ClinicalTrials.gov NCT01466686)*

Purpose

To compare the effects of

low dose fractionated radiation (LDFRT: 4x0.125 Gy, 4x0.25 Gy, 4x0.5 Gy)

versus

single dose radiation (0.5 Gy, 1 Gy, 2 Gy)

on

cytotoxicity of cisplatin and paclitaxel in cervix cancer cel lines

Materials and methods

- Human cervix cancer cell lines:

  SiHa HRS- *Wouters et al. Radiation Research (1996) 146, 399-413*

  CaSki

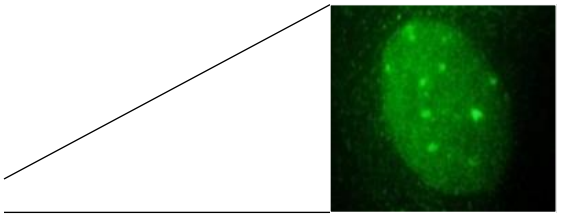
- Primary skin fibroblasts from patients with cervix cancer (CCU):

  HFIB2 HRS+ *Słonina et al. Int J Oncol Biol Phys (2014) 88, 369-376*

  HFIB29 HRS- *Słonina et al. Int J Oncol Biol Phys (2018) 100, 756-766*

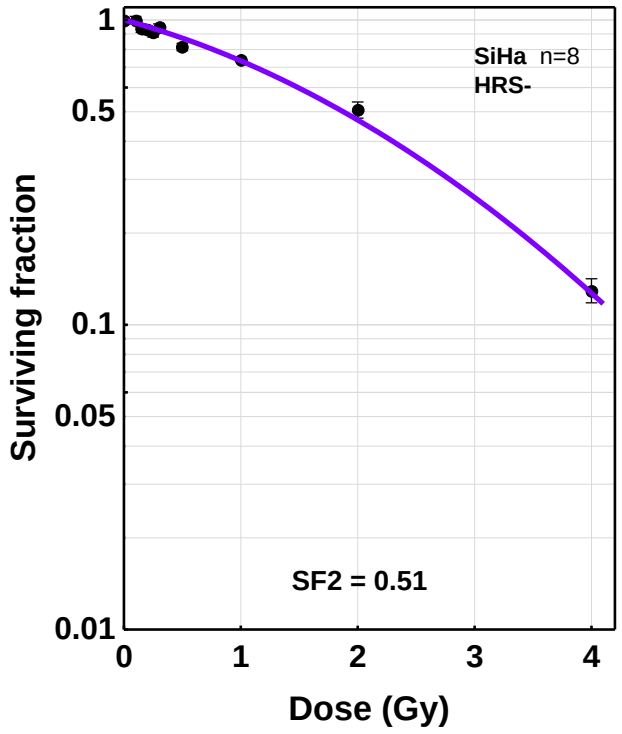
- Irradiation: Linac 6 MV X-rays

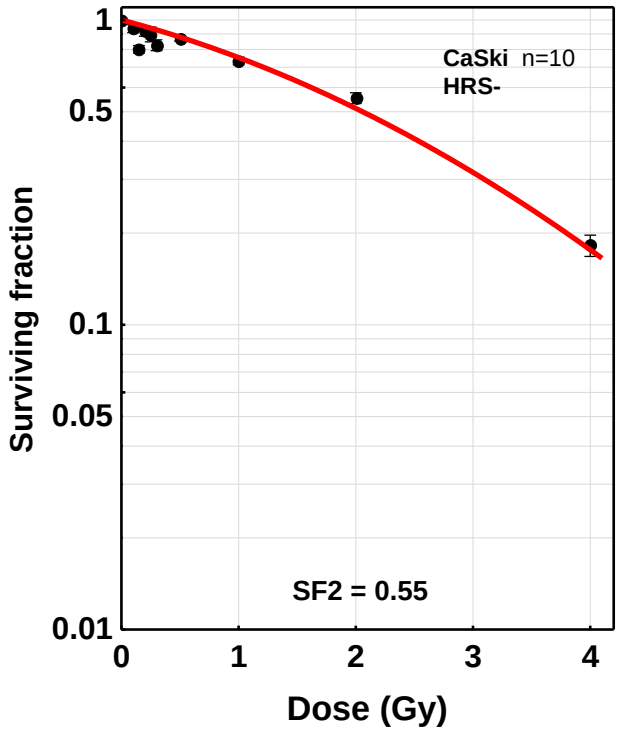
- Clonogenic assay: cell survival assay
- γH2AX assay: DNA damage assay



1 focus γH2AX = 1 DSB

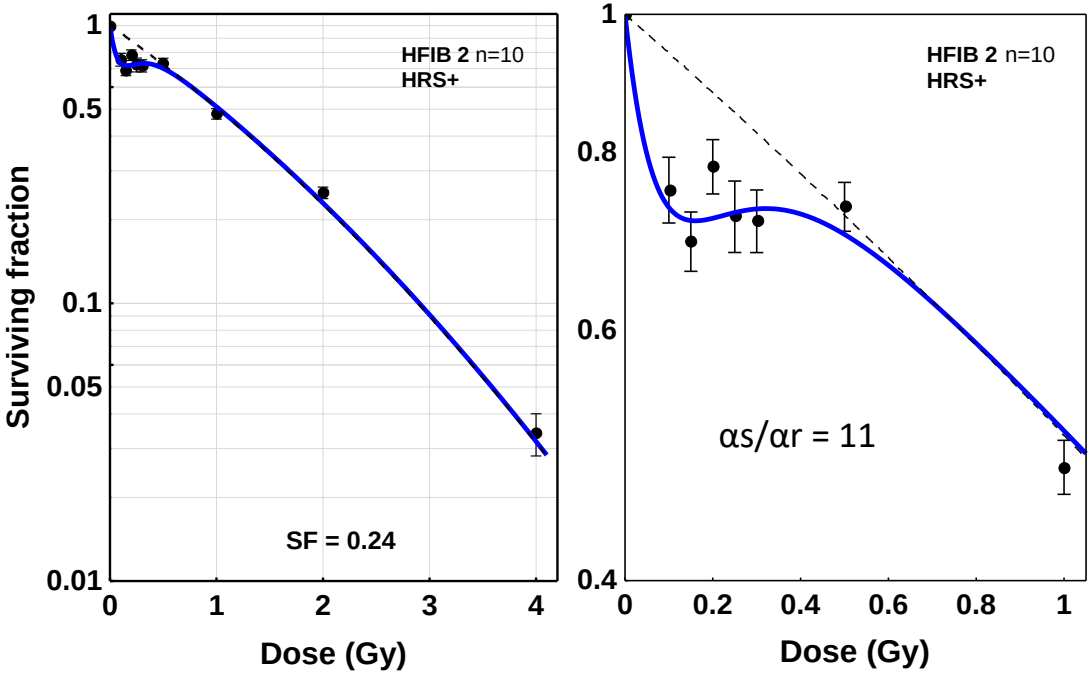
Survival of cervix cancer cells assessed by the flow cytometry-based clonogenic survival assay



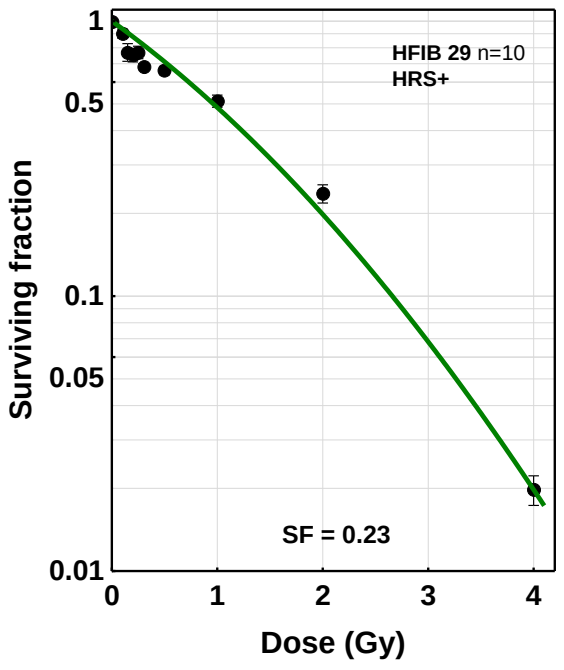


Lack of HRS phenomenon in SiHa and CaSki cells

Survival of normal fibroblasts of CCU patients assessed by the flow cytometry-based clonogenic survival



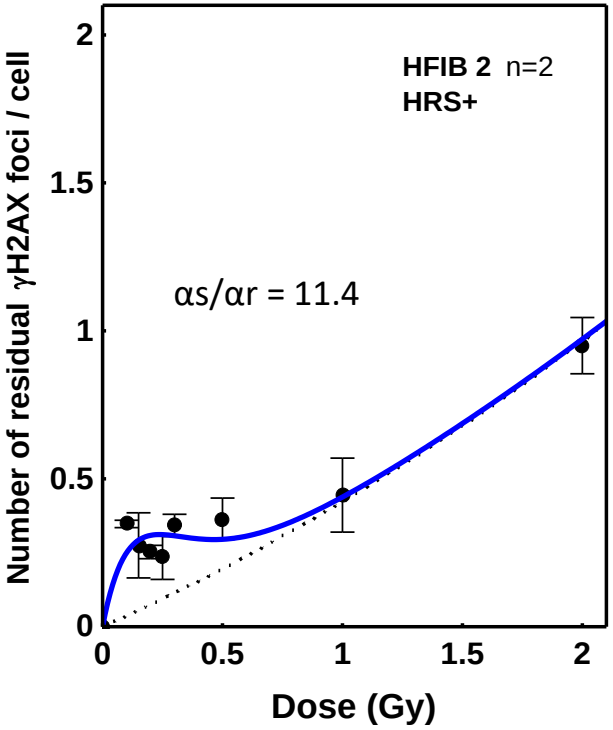
Presence of HRS in HFIB2



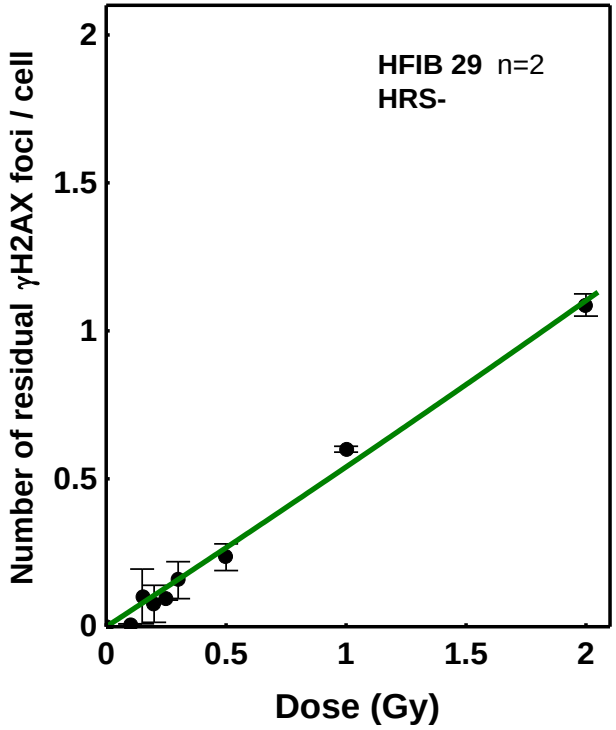
Lack of HRS in HFIB29

Słonina et al. Int J Oncol Biol Phys (2014) 88, 369-376

DNA damage in normal fibroblasts of CCU patients assessed by the γH2AX assay



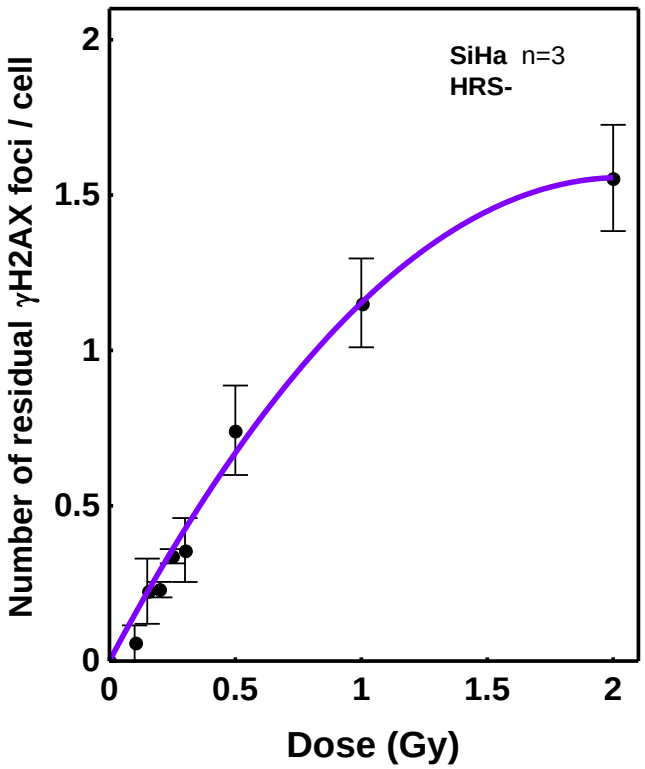
Presence of HRS in HFIB2

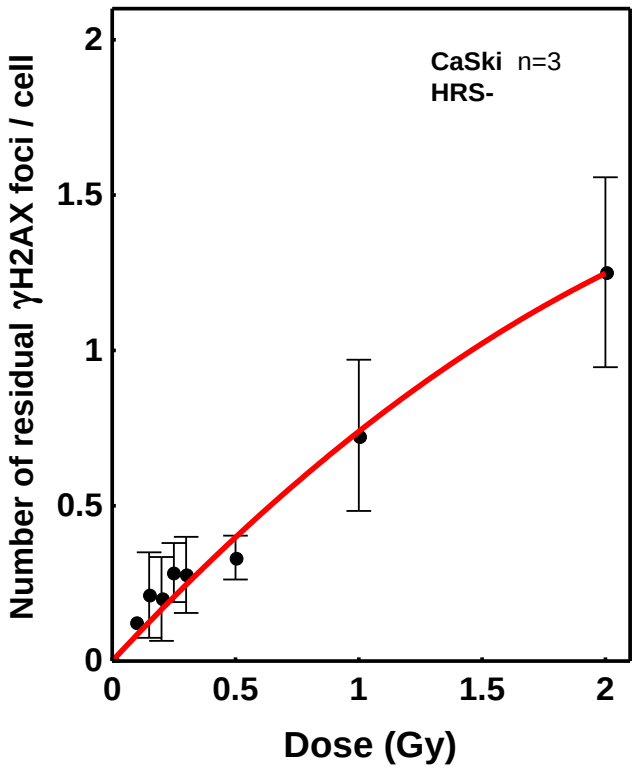


Lack of HRS in HFIB29

Słonina et al. Int J Oncol Biol Phys (2018) 100, 756-766

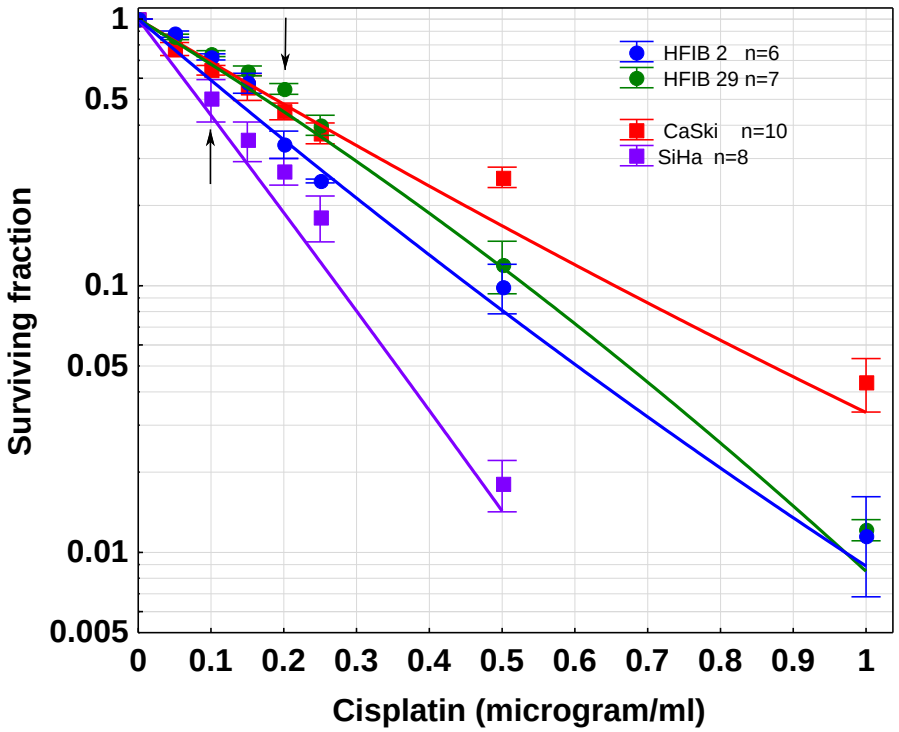
DNA damage in cervix cancer cells assessed by the γH2AX assay





Lack of HRS phenomenon in SiHa and CaSki cells

Effect of cisplatin on survival of cervix cancer cells and fibroblasts of CCU patients

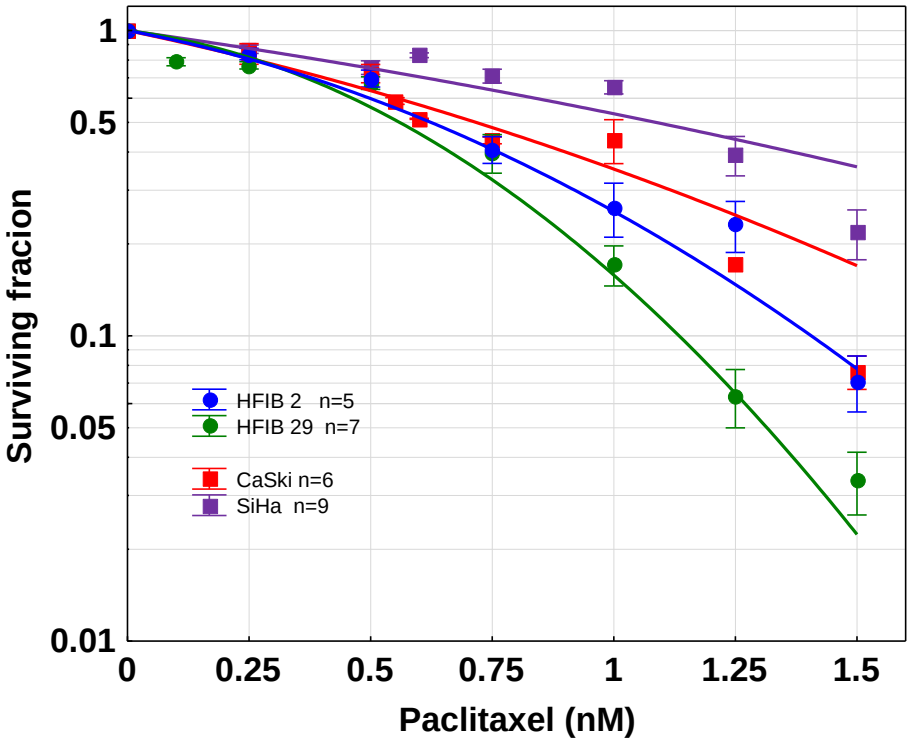


IC_{50} inhibitory concentration of cisplatin:

SiHa - 0.1 µg/ml
CaSki - 0.2 µg/ml
HFIB2 - 0.2 µg/ml
HFIB29 - 0.2 µg/ml

IC_{50} – is the concentration of drug in the cell culture medium, required to inhibit cell survival by 50%.

Effect of paclitaxel on survival of cervix cancer cells and fibroblasts of CCU patients

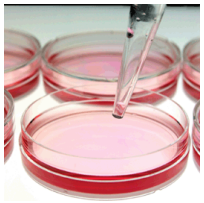
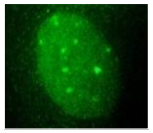
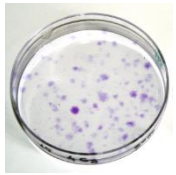
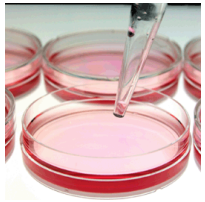
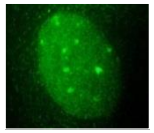
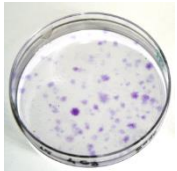


IC_{50} inhibitory concentration of paclitaxel:

SiHa - 1 nM
CaSki - 1 nM
HFIB2 - 0.75 nM
HFIB29 - 0.5 nM

IC_{50} – is the concentration of drug in the cell culture medium, required to inhibit cell survival by 50%.

Treatment protocol (cisplatin or paclitaxel plus irradiation)

Day 1	24h →	Day 2	24h →	Day 3	24h →	Day 4	Day 5	….Day 12-14
		C Cisplatin or Paclitaxel		0.5 Gy 1 Gy 2 Gy		 H2AX assay		 Clonogenic assay
		C Cisplatin or Paclitaxel		6h 0.125 Gy 0.125 Gy 0.25 Gy 0.25 Gy 0.5 Gy 0.5 Gy		6h 0.125 Gy 0.125 Gy 0.25 Gy 0.25 Gy 0.5 Gy 0.5 Gy	 H2AX assay	 Clonogenic assay

Conclusions

Low dose fractionated radiation (LDFR) potentiates cytotoxicity of cisplatin and paclitaxel in human cervix cancer cell lines irrespective of HRS status.

Cisplatin and paclitaxel enhancement ratios by radiation were higher with low dose fractionated radiation than with single dose radiation in cervix cancer cells and in HRS-positive fibroblasts from CCU patients.

Aknowledgment

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