Effect of low dose fractionated 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

LDFRT potentiates the cytotoxic effects of chemotherapy


LDFRT does not induce MDR1 protein responsible for multidrug-resistance

Molecular mechanisms underlying the process of chemopotentiation by LDFRT

- 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.

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 (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+  
  - HFIB29: HRS-

• 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

DNA damage in normal fibroblasts of CCU patients assessed by the $\gamma$H2AX assay

Presence of HRS in HFIB2  Lack of HRS in HFIB29

$\alpha_s/\alpha_r = 11.4$

DNA damage in cervix cancer cells assessed by the $\gamma$H2AX 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\textsubscript{50} inhibitory concentration of paclitaxel:
- SiHa - 1 nM
- CaSki - 1 nM
- HFIB2 - 0.75 nM
- HFIB29 - 0.5 nM

IC\textsubscript{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)

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<td>Cisplatin or Paclitaxel</td>
<td>0.5 Gy</td>
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<td>2 Gy</td>
<td><strong>H2AX assay</strong></td>
<td><strong>Clonogenic assay</strong></td>
<td><strong>H2AX assay</strong></td>
<td><strong>Clonogenic assay</strong></td>
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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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