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Cell Viability, DNA Damage And Relative Mitotic Arrest Dependence On Radiation Dose

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Introduction (1)

- Radiotherapy is one of the conventional cancer treatment therapies applied in oncology clinics. Ionising radiation effects on cancer cells and healthy tissues has to be thoroughly researched, for appropriate tumor treatment.

- Even though radiotherapy as a conventional treatment strategy is in clinical use, further development is needed to be done *in vitro* and *in vivo*. According to 3R principle, animal use can only be allowed if the goal can not be reached with *in vitro* experiments.

Introduction (2)

- Therefore *in vitro* experiments using cells were performed. Comet assay and clonogenic assay are frequently used methods for assesing ionising radiation impact on cells. Commet assay shows damage done to DNA structure and clonogenic assay is used to asses cell viability and proliferation.

- Aim of this study: apply clonogenic and Comet assays to asses damage done to DNA and cell proliferation.

- Materials and methods:
  - Chinese hamster ovary cell line.
  - Comet assay.
  - Clonogenic assay.

Cell plating

- Trypsinization:
  - Growing medium is removed
  - 1 ml PBS to wash cells
  - 2 ml 0.05% trypsin/EDTA solution for 3 minutes
  - 1 ml of growth medium containing FBS
- For irradiation:
  - 100 000 cells/dish
- For clonogenic cell viability assay:
  - 400 cells/dish

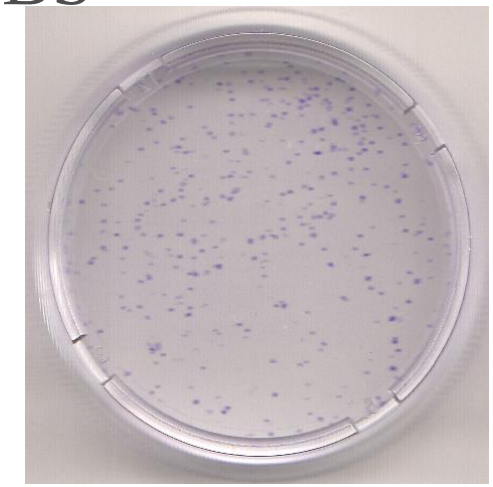


Fig. 1 Stained cell colonies using clonogenic assay

Irradiation procedure

- Varian Clinac DMX
- 6 MV energy photons
- Field size 10x10cm
- 3 Gy/min dose rate
- Doses 2-10 Gy

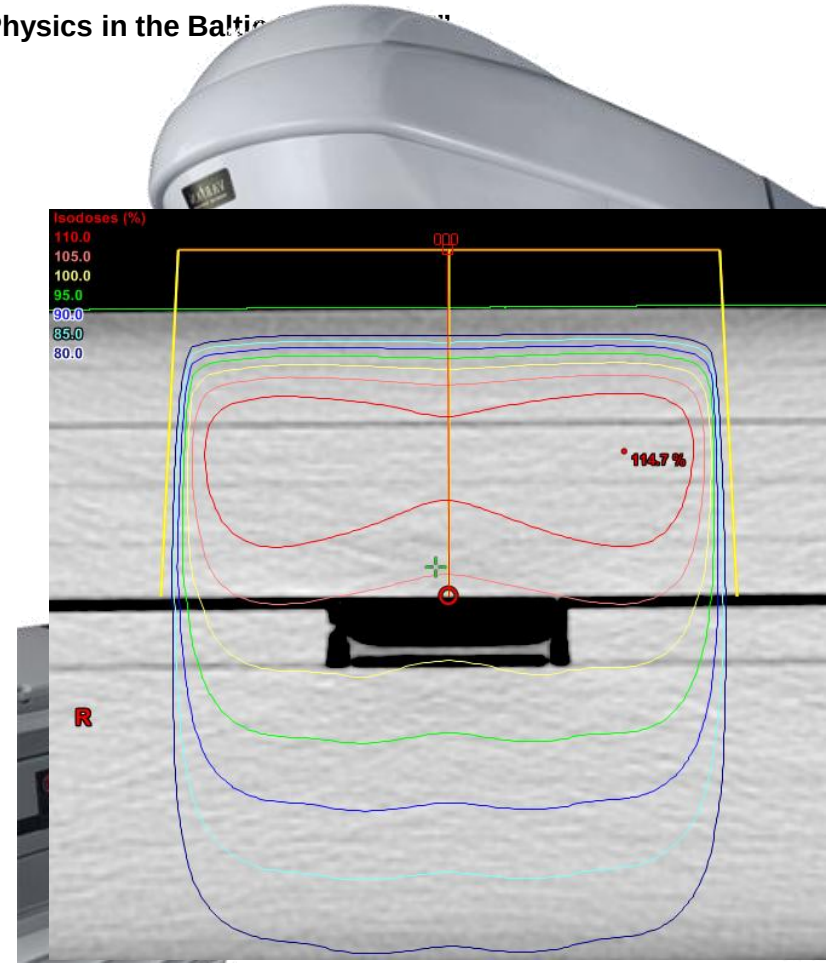


Fig. 3. Isodose distribution in PMMA plate.

- Petri dish (35x10 mm) placed inside 35x10 mm hole in 30x30x11 cm PMMA plastic
- 100% isodose at the base of the Petri dish.

Fig. 2. Linear accelerator used for irradiation.

Comet assay (1)

- Data is collected at the level of the individual cell.
- Assay can be done using relatively small numbers of cells per sample (<10,000)
- High sensitivity for detecting DNA damage
- Virtually any eukaryotic cell population is suitable for analysis

COMET assay (2)

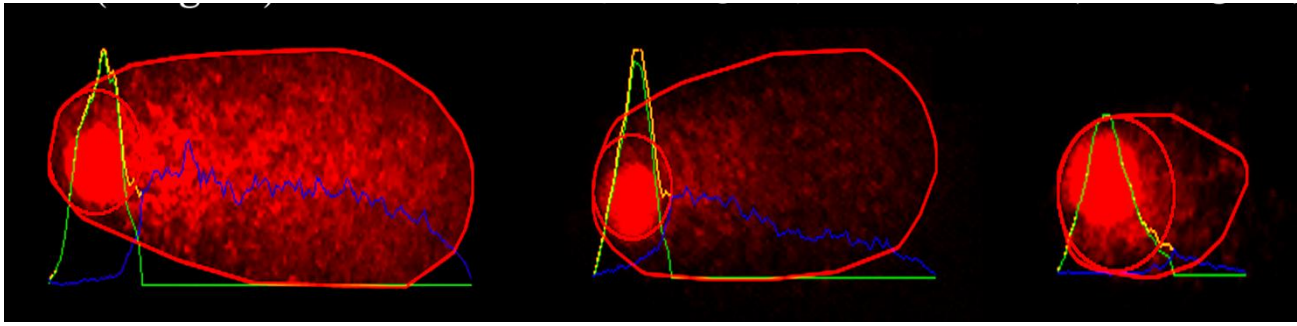


Fig. 4 Example of comet assay results

- Trypsinization
- 10 000 cell/sample
- 75µL, 37˚C, 0.5% LMPA/PBS solution
- Slides covered with NMA
- Lysing solution (pH = 10)
- Electrophoresis buffer (pH > 13), 20min.
- 0,74 V/cm, 300mA, 30 min.
- Neutralization buffer.
- 80 µL of 10 µmol ethidium bromide.

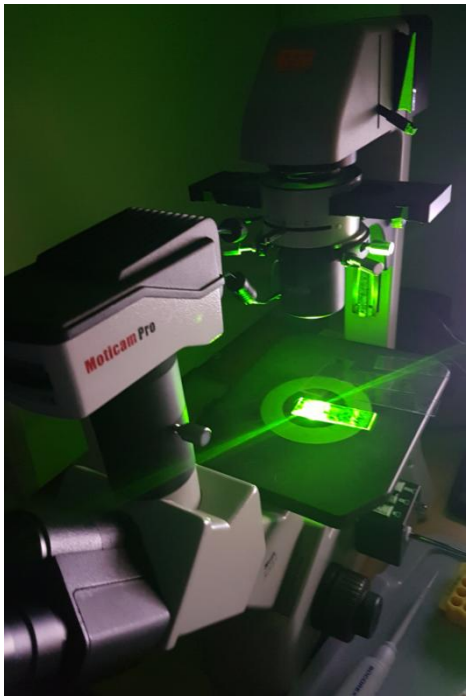
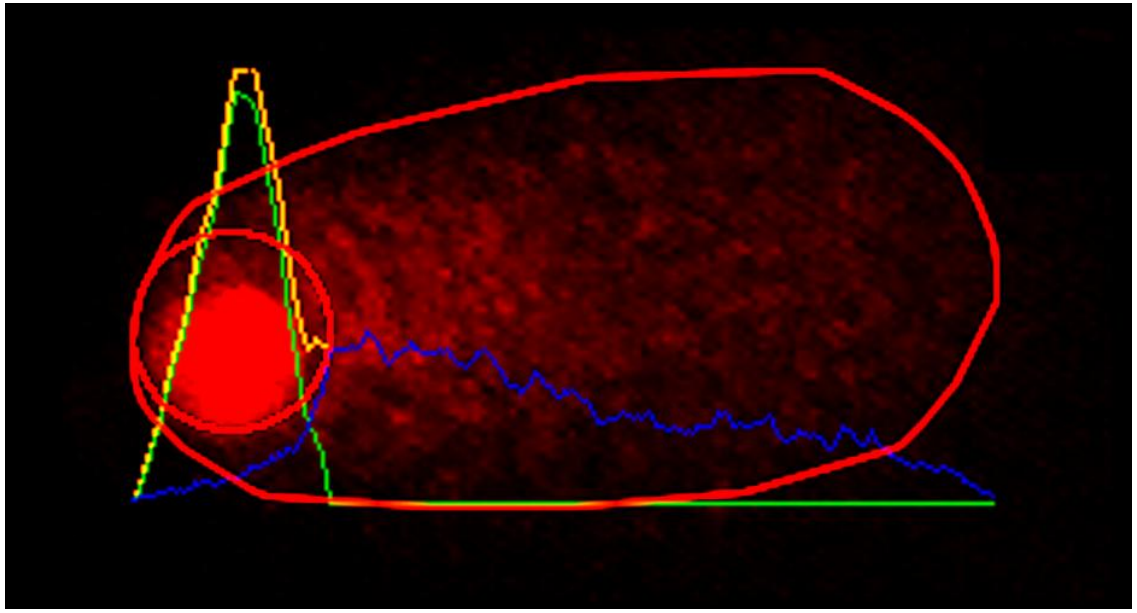


Fig. 5 Flourescence inverted microscope Motic AE31 used for comet assay assesment.

COMET assay (3)

- Analysis using OpenComet plugin in ImageJ.



Comet outline (red)

Comet profile(yellow)

Head profile(green)

Tail profile (blue)

Fig. 6 Example of comet assay anlysis using OpenComet plugin in ImageJ.

Results (1)
Comet assay

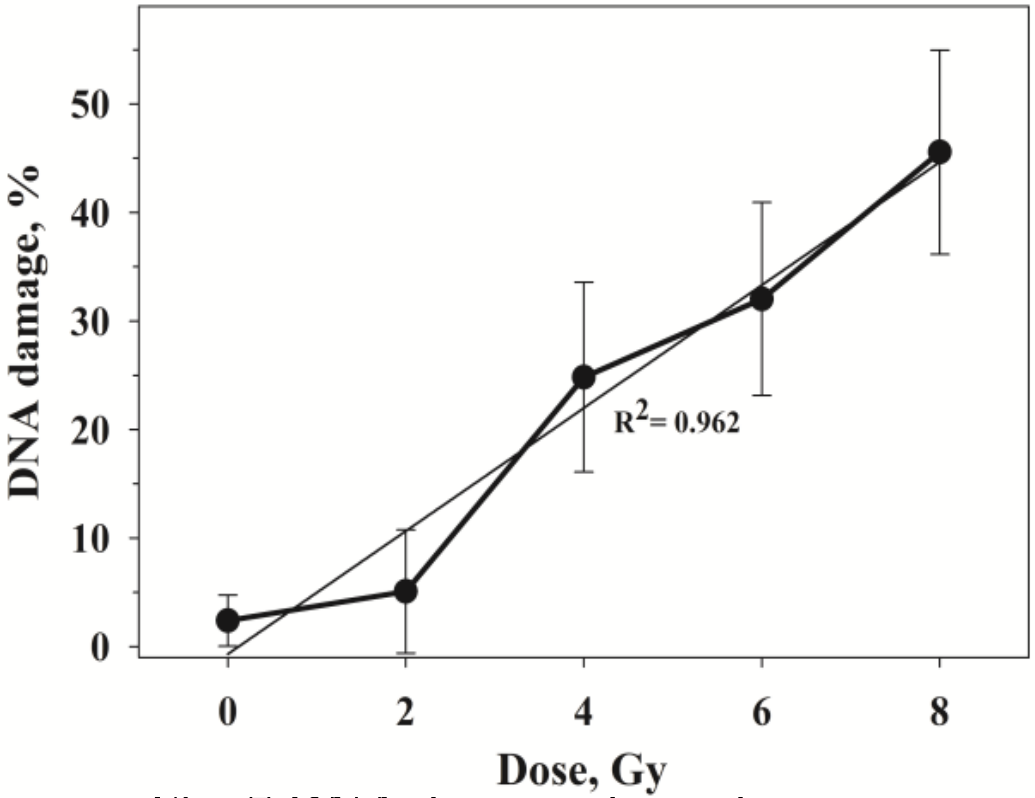


Fig. 7 DNA damage dependence on absorbed high energy x-ray dose analyzed with Comet assay.

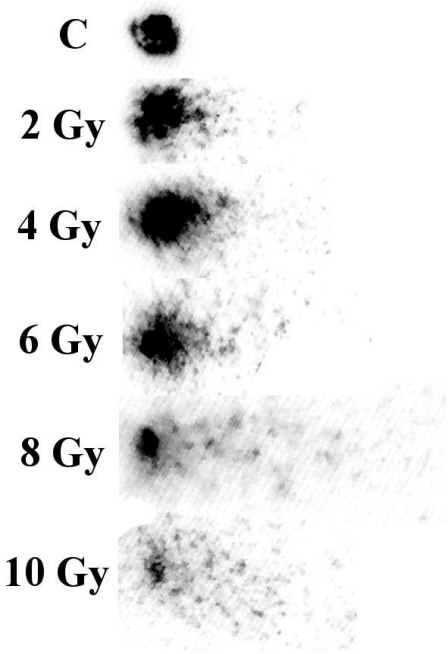


Fig. 8 Visualisation of DNA damage dependence on absorbed dose using comet assay.

Results (2)
Cell survival and average cell colony area

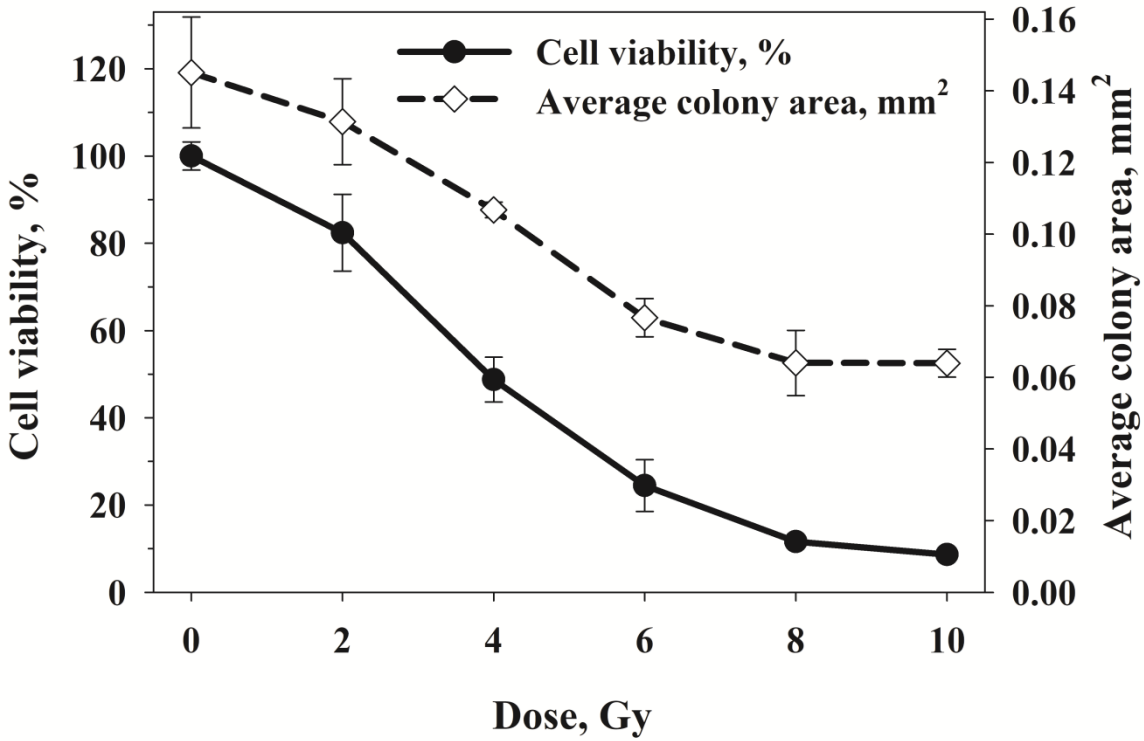


Fig. 9 Cell survival dependence on delivered dose and corresponding average cell colony area after irradiation. ;

Conclusions

- Cells that experience repairable DNA damage can undergo delayed mitosis. Threshold dose of 6 Gy for cell irradiation at which maximal delayed mitosis effect with initiation of ~30 % of DNA damage on surviving cell fraction was observed.

Conclusions

- Our results indirectly showed that mitotic arrest after cell irradiation to ≥ 6 Gy was the same for the surviving fraction of cells.
- The ≥ 6 Gy threshold dose hypothesis does not apply for cell viability, since it was observed that after cell irradiation to doses from the interval of 6-10 Gy the cell viability decreases from 24±6% to 9±1% respectively. Moreover this hypothesis also does not apply to DNA damage

Thank you for attention!