Analysis of high-LET radiation-induced HPRT mutations in mammalian cells

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Plan of the presentation

1. Aim of the project
2. High-LET radiation
3. HPRT gene
4. Work description
5. Equipment
6. Results
7. Conclusions

Aim of the project

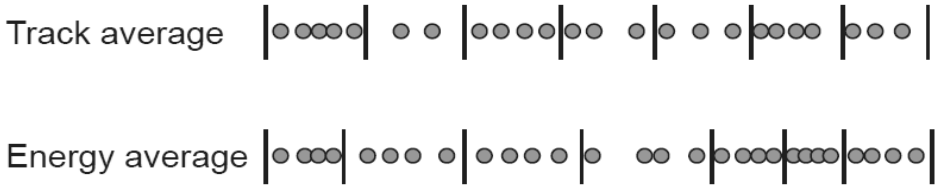
- Structural analysis of HPRT mutations caused by irradiation
- Analysis of partial and/or total deletions of exons using the PCR method
- Identification and distinction of the changes in the genetic material of the cells after γ and ^{18}O irradiation

FINAL AIM:

Clarification of molecular mechanisms of mutagenic effects induced by different types of ionizing radiation in cells in radiation genetics investigations

High-LET radiation

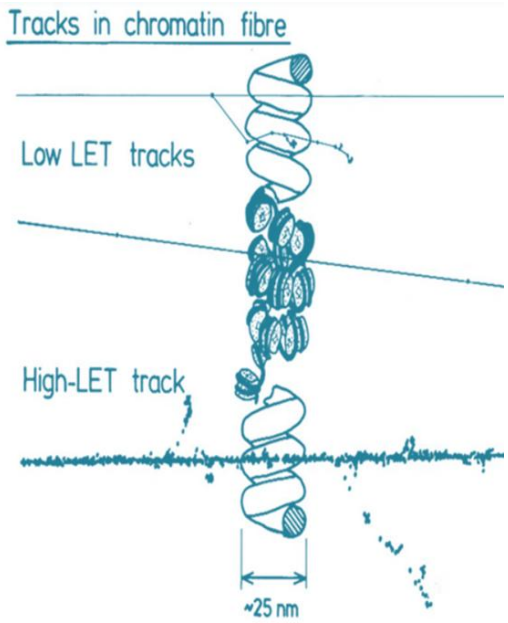
LET = Average energy deposited per unit length of track (keV/μm)



$LET_{\gamma} = 0{,}3$ keV/μm

$LET_{^{18}O} = 115$ keV/μm

high-LET (high linear energy transfer) – densely ionising radiation, eg. heavy ions, α particles, have a greater biological effectiveness than low-LET.

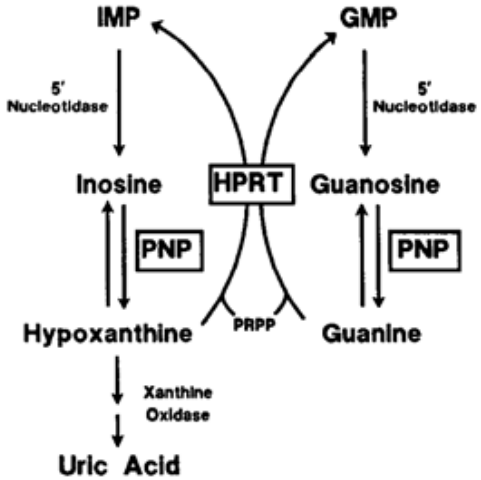


1. Eric J. Hall, Amato J. Giaccia, *Radiobiology for the radiologist*, 7th edition, Philadelphia 2012, ISBN 978-1-60831-193-4
2. Dudley T. Goodhead, Mechanisms for the Biological Effectiveness of High-LET Radiations, J. Radiat. Res., 40: SUPPL., 1-13 (1999)

HPRT gene

MATRSPSVVISDDEPGYDLDLFCIPNHYVEDLEKVFIPHGVIMDRTERLARDVMKEMGGH
HIVALCVLKGGYKFFADLLDYIKALNRNSDRSIPMTVDFIRLKSYCNDQSTGDIKVIGGD
DLSTLTGKNVLIVEDIIDTGKTMQTLLSLVKRYNLKMVKVASLLVKRTSRSVGYRPDFVG
FEIPDKFVVGYALDYNEYFRDLNHICVISETGKAKYKA

- 9 exons
- 36 kB of DNA
- often used as a target for mutation studies in vitro because of the ease of selection of forward and reverse mutants

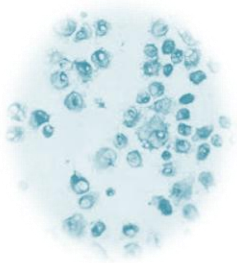


1. Rossiter, B.J.F.; Fuscoe, J.C.; Muzny, D.M.; Fox, M.; Caskey, C.T. The Chinese hamster HPRT gene: Restriction map, sequence analysis, and multiplex PCR deletion screen, *Genomics*. vol. 9 (2), 1991, 247–256
2. Stout, J.T.; Caskey, C.T. HPRT: Gene Structure, Expression, and Mutation, *Annual Review of Genetics*. vol. 19 (1), 1985, 127–148.

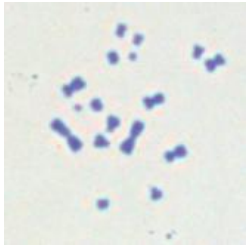
Work description

cell culture recultivation

V79



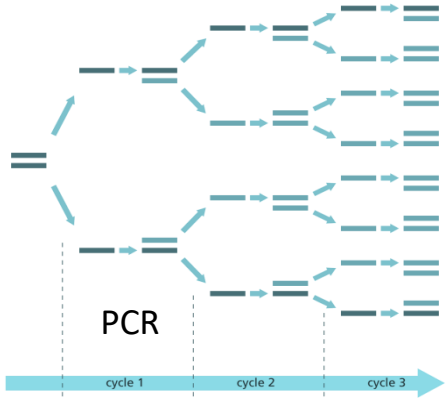
cell isolation

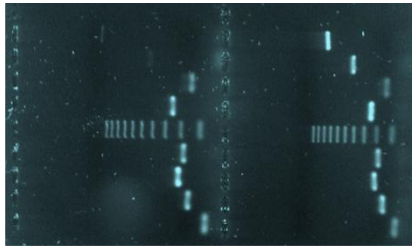


DNA isolation

chromosomal aberration analysis

DNA concentration measurements (dilution)

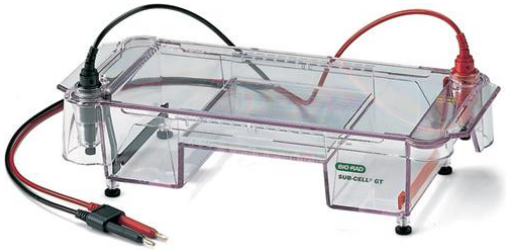




gel electrophoresis and analysis

Equipment

Real-Time PCR Detection System (Bio-Rad)



Sub-Cell GT Cell (Bio-Rad)

Gel Doc EZ System (Bio-Rad)

Results

Table 1. Results of real-time PCR performed on the mutant DNA.

Irradiation	Dose	No. of mutant	Ex1	Ex2	Ex3	Ex4	Ex5	Ex6	Ex7,8	Ex9
γ	0,5 Gy	5A								
γ	0,5 Gy	8A								
γ	0,5 Gy	7A								
γ	0,5 Gy	10A								
γ	2 Gy	1A								
γ	2 Gy	6A								
γ	2 Gy	1Б								
γ	2 Gy	7Б								
^{18}O	0,5 Gy	3Б								
^{18}O	0,5 Gy	2A								
^{18}O	0,5 Gy	16A								
^{18}O	0,5 Gy	3A								
^{18}O	2 Gy	3A								
^{18}O	2 Gy	3B								
^{18}O	2 Gy	5Б								
^{18}O	2 Gy	10Б								

Legend:

exon amplified

exon lost

Results – gel electrophoresis

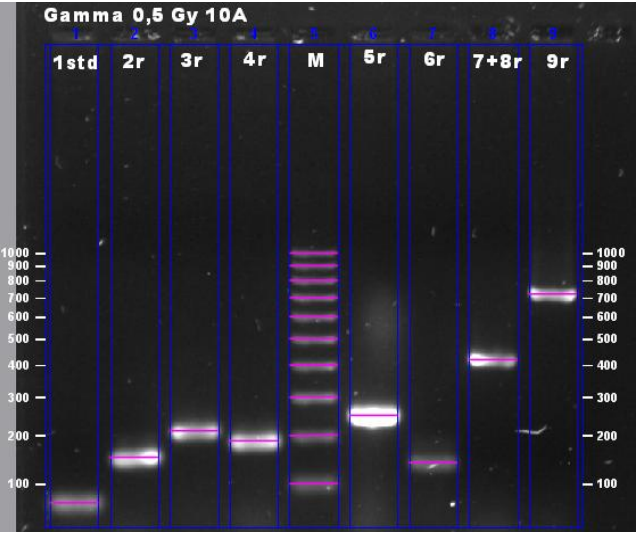


Fig. 1. Mutant 10A – all exons present.

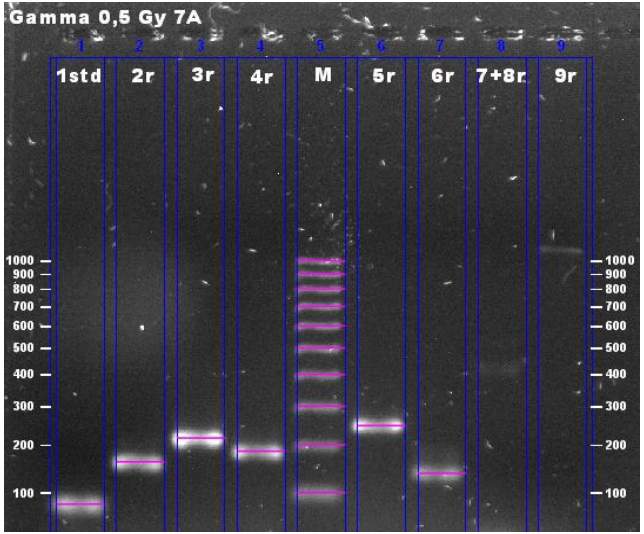


Fig. 2. Mutant 7A – partial deletion.

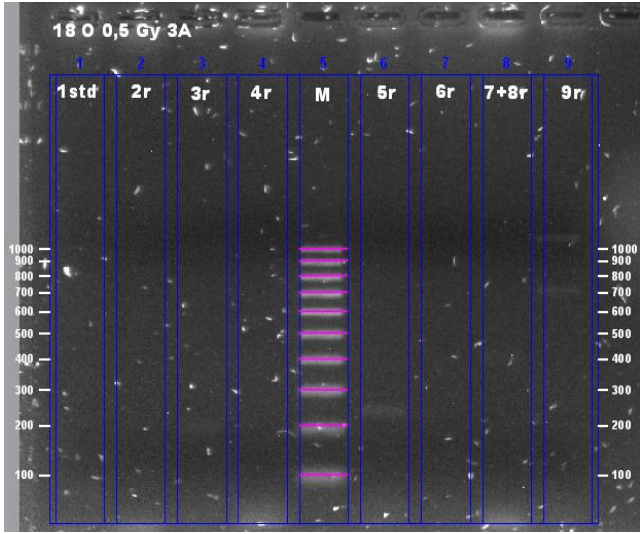
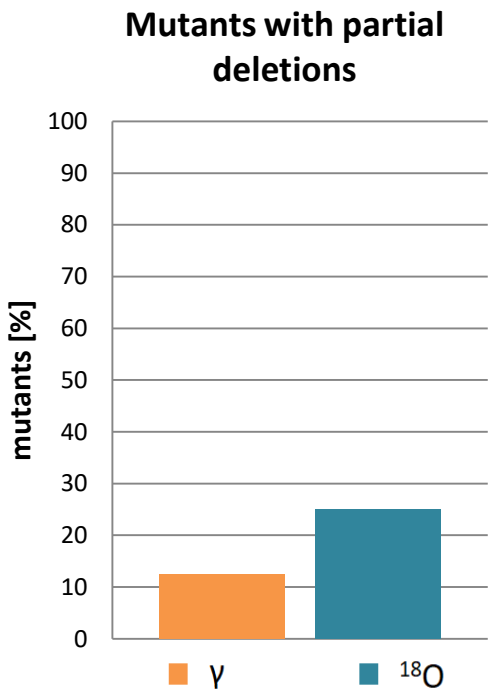
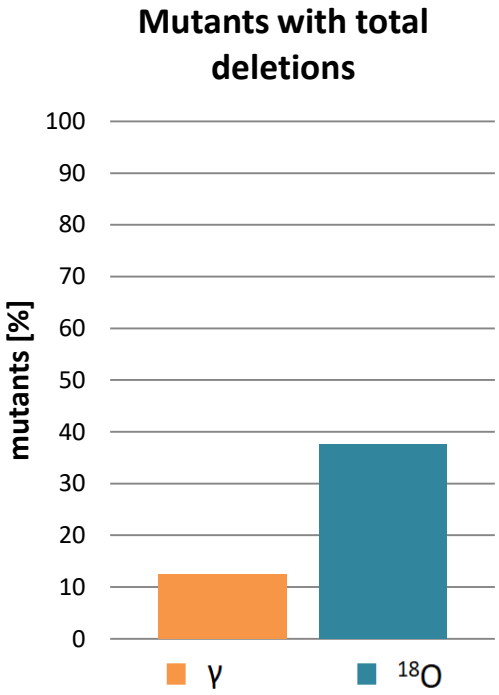
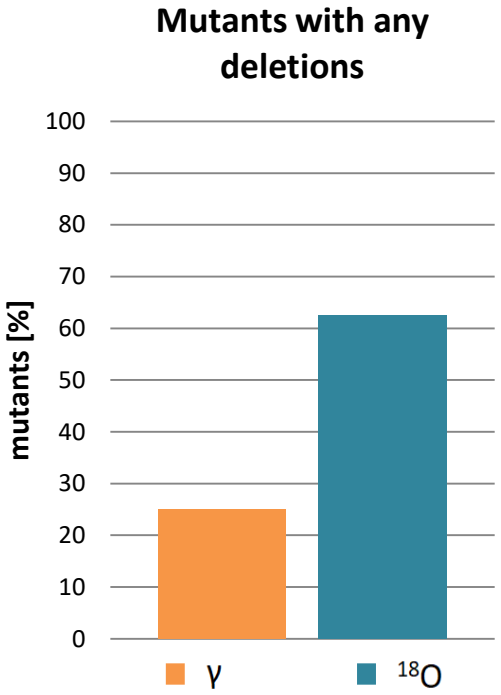


Fig. 3. Mutant 3A – total deletion.

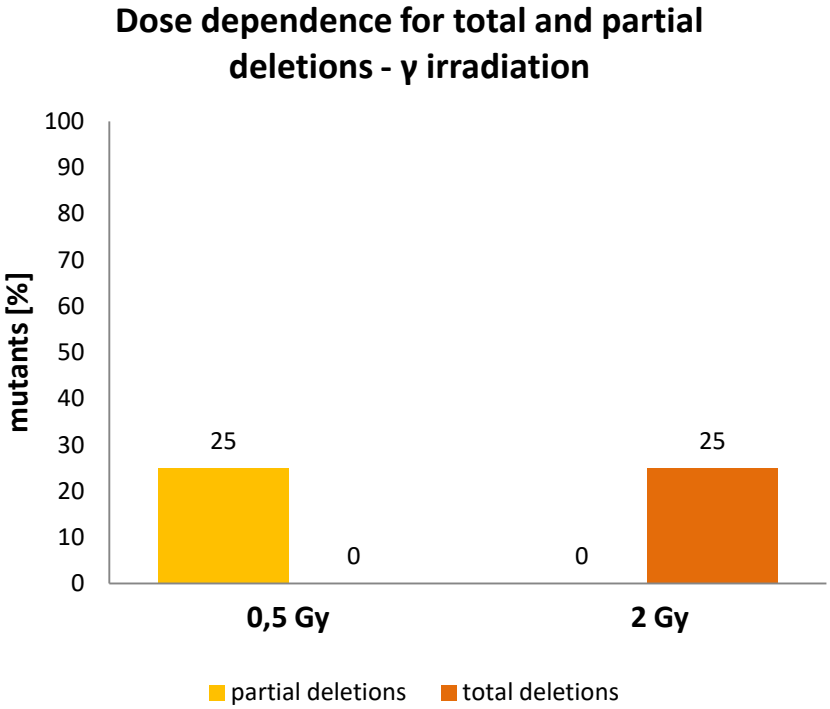
Results

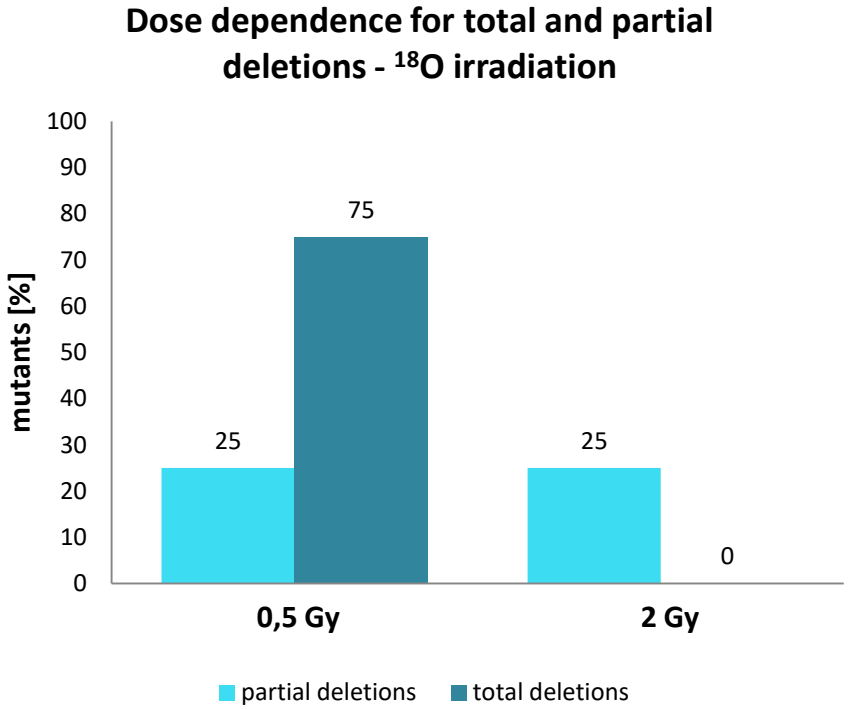

Mutants with partial deletions
mutants [%]
100
90
80
70
60
50
40
30
20
10
0
γ
18O


Mutants with total deletions
mutants [%]
100
90
80
70
60
50
40
30
20
10
0
γ
18O


Mutants with any deletions
mutants [%]
100
90
80
70
60
50
40
30
20
10
0
γ
18O

Results


Dose dependence for total and partial deletions - γ irradiation
mutants [%]
100
90
80
70
60
50
40
30
20
10
0
25
0
0
25
0,5 Gy
2 Gy
partial deletions
total deletions


Dose dependence for total and partial deletions - ^{18}O irradiation
mutants [%]
100
90
80
70
60
50
40
30
20
10
0
25
75
25
0
0,5 Gy
2 Gy
partial deletions
total deletions

Conclusions

- PCR and gel electrophoresis are excellent methods in monitoring damages of genetic material.
- Both types of irradiation and two studied doses cause deletions in the DNA of V79 cell line.
- ^{18}O appears to cause two times more exon deletions in the DNA.
  - Perhaps it is linked with higher LET value (or ROS).
- There is no apparent dose-type of deletion relationship for the γ irradiated cells.
- 0,5 Gy dose of ^{18}O seems to cause more damage than 2 Gy.
  - This may be due to the fact that 2 Gy of accelereted oxygen ions kill cells right after the irradiation.

Thank you for your attention