Radiation genetics, epigenetics and effects on clock genes

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Radiation genetics = target theory
Independently developed in 1949
by NV Timofeev-Ressovsky & DE Lea

- Everything happens in the directly irradiated cell & mutation induction occurs at the radiation-damaged sites (targets)
- The yield of mutations is proportional to the amount of initial DNA damage & efficiency of its repair, \textit{i.e.} depends on the dose, dose-rate & type of irradiation
- The risk of exposure to ionising radiation is described by the Linear No-Threshold Model
Radiation-induced genomic instability in somatic cells

Yield of chromatid aberrations in MCF10A cells

Delayed mutations occur many cell divisions after exposure

Everything happens in the directly irradiated cell & mutation induction occurs at the radiation-damaged sites

What about the germline?

How to analyse?

Instability in the non-exposed offspring of irradiated parents

Mutation frequency

F₀

F₁

F₂

Mutant

Are they unstable?
Mouse Expanded Simple Tandem Repeat (ESTR) loci

- 4-10 bp repeats, 100 bp - 20 kb arrays, non-coding
- Very spontaneous mutation rate (up to 15% per gamete)
- Mutations result in the loss/gain of repeats
ESTR mutation detection in the germline & somatic tissues

Pedigree approach

Single-molecule PCR approach

Father

Maternal
mutation

Paternal
mutations

Mutants

Mother
0.5 Gy of fission neutrons

Let’s go transgenerational…

From: Dubrova et al., 2000, Nature 405, 37
Transgenerational germline instability in the F₁ offspring of CBA/H male mice exposed to 0.5 Gy of fission neutrons

The non-exposed offspring of irradiated parents are unstable

Is transgenerational instability strain-specific?
Fission neutrons, 0.4 Gy: CBA/H; C57BL/6
Acute X-rays, 2 Gy: CBA/H
Acute X-rays, 1 Gy: BALB/c

From: Barber et al., 2002, PNAS 99, 6877-82
Transgenerational instability in three inbred mouse strains

ESTR mutation rates are elevated in both generations of all inbred strains

From: Barber et al., 2002, PNAS 99, 6877-82
Is transgenerational instability tissue-specific?
Transgenerational instability in the germline & somatic tissues

ESTR mutation rates are equally elevated in the germline & somatic tissues

From: Barber et al., 2006, Oncogene 25, 7336-42; 2009, Mutat Res 664, 6-12
Is transgenerational instability specific for tandem repeat loci?
Transgenerational instability at the mouse *hprt* locus

Chromosome aberrations in the F$_1$ offspring of irradiated rats

A genome-wide destabilisation

*hprt* is X-linked gene

From: Barber et al., 2006, *Oncogene* 25, 7336-42

For how long can a transgenerational signal survive in the irradiated males?
Adult

<1 week

Sperm

Instability?
3 weeks
Spermatids
Instability?
6 weeks

Adult

♀♂

Spermatogonia

Instability?
Primordial stem cells

Instability?

in utero
Transgenerational effects manifest in the offspring regardless the stage of paternal irradiation. Stage of paternal irradiation:

- 1 week
- 6 weeks
- In utero

From: Barber et al., 2002, PNAS 99, 6877-82; 2006, Oncogene 25, 7336-42; 2009, Mutat Res 664, 6-12; Hatch et al., 2007, Oncogene, 26, 4720-4
Can paternal exposure to chemical mutagens destabilise the F$_1$ genomes?
Anticancer drug cyclophosphamide, CPP
- alkylated monoadducts & crosslinks
- results in base substitutions
- crosslinks can result in DSBs after replication/repair

Alkylating agent ethynitosurea, ENU
- mostly base damage
- results in base substitutions
- ~ no ENU-induced DSBs

Anticancer drug mitomycin C, MMC
- alkylated monoadducts & crosslinks
- base substitutions
- crosslinks can result in DSBs

Anticancer drug procarbazine, PCH
- alkylated monoadducts
- free radical species
- base substitutions & SSBs
Instability signal is initiated by a generalised DNA damage

From: Barber et al., 2002, *PNAS* 99, 6877-82
Dubrova et al., 2008, *Environ Mol Mutagen* 49, 308-11
Glen, Dubrova 2012, *PNAS* 109, 2984
Is transgenerational instability sex-specific?
The offspring of irradiated females are stable.

Irradiated *in utero* versus Adult irradiation

Mechanisms
Some back of the envelope exercises...

~ 1,000 genes are involved in maintaining genome stability in mammals (DNA repair, apoptosis, cell cycle arrest etc)

max spontaneous mutation rate 10^{-6} per locus

exposure to 1 Gy of X-rays results in ~ a 3-fold increase in mutation rate

if ANY radiation-induced mutation at ANY of 1,000 genes is DOMINANT and can substantially compromise the genome stability, then

\[ 1000 \times 3 \times 10^{-6} = 0.3 \% \] of the F_1 offspring should be unstable

according to our data ~100% of the F_1 offspring of irradiated males are unstable

The mechanisms must be epigenetic
Initiation of an epigenetic instability signal in the directly exposed male germ cells

Transmission of an epigenetic instability signal to the offspring & its manifestation
Measuring DNA damage \textit{in vivo}

The alkaline Comet assay

Mostly single-strand DNA breaks + some DNA adducts

The $\gamma$H2AX assay

Double-strand DNA breaks only
Endogenous DNA damage in controls & the F₁ offspring of irradiated males

Single-strand DNA breaks
Comet assay, bone marrow

Double-strand DNA breaks
γ-H2AX assay, spleen

From: Barber et al., 2006, Oncogene 25, 7336-42
DNA repair in the $F_1$ offspring of irradiated males

The efficiency of DNA in the $F_1$ offspring is not compromised

From: Barber et al., 2006, *Oncogene* 25, 7336-42
Oxidative stress

DNA damage:
- modified bases
- single-strand breaks
- double-strand breaks

Hallmark:
Accumulation of oxidatively damaged nucleotides in DNA

The efficiency of DNA in the F₁ offspring is OK
No sign of oxidative stress in the F₁ offspring
What else?

From: Barber *et al.*, 2006, *Oncogene* 25, 7336-42
Transcriptome analysis of transgenerational effects

1 Gy of acute X-rays

CBA/Ca

F₀

♂ ♀

F₁

BALB/c

F₀

♂ ♀

F₁

RNA extraction

Kidney

Liver

Spleen

Brain

NimbleGen 12x135K expression arrays:
- 135,000 probes per array; 45-60mer long
- Complete coverage of the mouse transcriptome (42,576 transcripts)
- 3-4 probes per transcript
- 12 arrays per slide
Probabilities for the effects of paternal irradiation on F₁ gene expression

GO categories:
- GO:0048511 Rhythmic process, 6 genes  \( P = 1.25 \times 10^{-9} \)
- GO:0007623 Circadian rhythm, 5 genes  \( P = 1.52 \times 10^{-7} \)
- GO:0006355 Regulation of transcription, DNA-dependent, 11 genes  \( P = 1.62 \times 10^{-6} \)

FDR < .05; 56 transcripts
Compromised gene expression in the F₁ offspring

-6 -5 -4 -3 -2 -1 0 1 2 3

Ratio F₁/control, log₂

-6 -5 -4 -3 -2 -1 0 1 2 3

Dbp  Per2  Per3  Tef  Nr1d2  Mtf1  Lhx2  Nfil3  Ppard  Arntl  Npas2

down-regulated

up-regulated
Circadian transcriptome & circadian metabolism in mice

Circadian transcripts in mouse liver

From: Maywood et al., 2007, Cold Spring Harb Symp Quant Biol 72, 85
Akhtar et al., 2002, Curr Biol 12, 540
And so what?
Incidence of skin tumour in the offspring of irradiated male mice

From: Vorobtsova et al., 1993, Mutat. Res. 287, 207-216
Transgenerational effects in the children of irradiated parents

Childhood cancer survivors

From: Tawn et al., 2005, Mutat Res 523, 198-206; Aghajanyan & Suskov, 2009, Mutat Res 523, 52-7

Chernobyl clean-up workers

Unstable?
Experiment one:
Male mice exposed to 10 – 100 cGy acute $\gamma$-rays or 100 cGy chronic $\gamma$-rays

Experiment two:
Male mice exposed to clinically-relevant doses of 3 anticancer drugs:
Cyclophosphamide
Mitomycin C
Procarbazine

Sperm, brain, bone marrow
Instability signal is triggered by a stress-like response in irradiated cells.

From: Glen, Dubrova 2012, PNAS 109, 2984
High-dose acute paternal exposure to a number of mutagens can significantly destabilise the genomes of their offspring.

Transgenerational instability is a genome-wide phenomenon which affects the frequency of chromosome aberrations and gene mutations.

Transgenerational instability is triggered in the directly exposed germ cells by a stress-like response to a generalised DNA damage.

Transgenerational instability is attributed to the presence of a persistent subset of endogenous DNA lesions.

Transgenerational instability is attributed to the epigenetic changes affecting the expression of a subset of genes, involved in rhythmic process & regulation of transcription.

Transgenerational instability may represent an important component of the long-term genetic risk of human exposure to mutagens, but we need HUMAN data to prove it!
Acknowledgements

- **Dubrova’s lab**
  Ruth Barber  Colin Glen  Safeer Mughal  Andre Gomes
  Robert Hardwick  Carole Yauk  Mariel Voutounou  Tim Hatch
  Dominic Kelly  Peter Hickenbotham  Morag Shanks  Carles Vilarino-Guell
  Karen Monger  Bruno Gutierrez  Karen Burr  Julia Brown
  Natalya Topchiy  Isabelle Roux  Peter Black  Demetria Pavlou
  Hamdy Ali Abouzeid

- **MRC Radiation and Genome Stability Unit, Harwell, UK**
  Mark Plumb  Emma Boulton  Jan Fennelly  Dudley Goodhead

- **Department of Cancer Studies and Molecular Medicine, University of Leicester, UK**
  George “Don” Jones  Gabriela Almeida  [Comet Assay]
  Alexander Rubanovich  Andrey Myazin  [Chronic irradiation]

- **NI Vavilov Institute of General Genetics, Moscow, Russia**
  Leonid Zhavoronkov  Yuri Semin  Albert Brovin  [Chronic irradiation]
  Valentina Glushakova  Valentina Posadskaya  Olga Izmet’eva

- **Medical Radiological Research Centre, Obninsk, Russia**
  Peter de Boer  Alwin Derijck  Godfried van der Heijden  [Sperm irradiation]

- **MRC Toxicology Unit, Leicester, UK**
  Andy Smith  [Anticancer drugs]

- **Centre for Molecular Genetics and Toxicology, University of Wales, Swansea, UK**
  George Johnson  James Parry  [Hprt assay]

- **Catholic University of Nijmegen, The Netherlands**
  Peter de Boer  Alwin Derijck  Godfried van der Heijden  [γH2AX assay]

- **Gray Cancer Institute, Northwood, UK**
  Kai Rothkamm