Cellular Defenses against Radiation Injury
DNA DAMAGE AND HUMAN DISEASE

EXOGENOUS FACTORS
- Base damage
- Bulky adducts
- Crosslinks

ENDOGENOUS FACTORS
- DNA breaks
- Mispaired bases

Effects:
- Toxicity
- Genomic instability
- Homeostasis

Conditions:
- Cancer
- Neurodegeneration
- Developmental problems
- Ageing
The DNA Damage Response

A network of pathways that protects against DNA damage

transcription responses  
cell cycle arrest  
DNA repair  
apoptosis  
chromatin remodeling  
Damage bypass

RNA splicing*  
translation responses **  
mitochondrial responses

DNA DAMAGE RESPONSE 2

SENSORS
RAD50
MRE11
NBS1...

TRANSDUCERS
ATM, ATR
53BP1, MDC1
BRCA1....

EFFECTORS
Chk1, Chk2
p53......

DNA Damage signalling
Kinases
Phosphatases
Ubiquitin ligases
Dubs etc.

Ac
P
Me
Ub
SUMO

transcription responses
DNA repair
apoptosis
chromatin remodeling
Damage bypass

RNA splicing*
translation responses **
mitochondrial responses

SUMMARY 1 (DDR)

- Maintenance of genomic integrity relies on functional DDR
- DDR encompasses initial DNA damage sensing up to translation control
- DDR is tightly regulated by posttranslational modifications
- The number of DDR genes is rapidly increasing and thereby the number of potential risk factors that contribute to DNA damage mediated disease.
- Factors related to human disease (cancer)
  - Cell cycle checkpoints/ apoptosis
  - DNA repair factors and chromatin remodelers
  - Factors involved in DNA damage signaling
Programmed DSBs

- V(D) J recombination
- Class-switch recombination
- Meiosis

Accidental DSBs

- DNA replication and transcription
- Ionizing Radiations
  - DNA base damage
  - DNA strand breaks
  - Clustered DNA damage

Goodarzi et al, Advances in Genetics, 2013
Recruitment of XPG to local UV damage

Recruitment of 53BP1 to laser induced damage
Double-strand break (DSB)

γH2AX

MRN

ATM

Cell cycle regulation and DSB repair

antibody

ATM

P
CHROMATIN AND THE DSB RESPONSE 2

DSB

Phosphorylation H2Ax

Amplification of signal

Focus
Local concentration of protein
Double-strand break (DSB)

Cell cycle regulation and DSB repair

CHROMATIN AND THE DSB RESPONSE 4

Luijsterburg et al., EMBO J. (2012)
DSB-REPAIR PATHWAYS

non-homologous endjoining

homologous recombination

• G1, S, G2

• Late S, G2
DSB-REPAIR PATHWAYS

non-homologous endjoining

• G1, S, G2

homologous recombination

• Late S, G2

sister chromatid
NON-HOMOLOGOUS ENDJOINING

Canonical NEHJ
fast
NON-HOMOLOGOUS ENDJOINING

Canonical NEHJ
fast

Alternative NEHJ
slow

SCID patients: XLF, Ligase IV, XRCC4, Artemis and DNA-PKcs
Ataxia telangiectasia patients

van der Burg et al., 2009
MODEL FOR RECOMBINATION (SIMPLE)

Presynaptic: resection

Synaptic: strand invasion and DNA synthesis

Postsynaptic: resolution
BRCA2: ESSENTIAL FOR RAD51 FILAMENT FORMATION

DSB → 5' to 3' end resection → Assemble of the RAD51 presynaptic filament by BRCA1–PALB2–BRCA2 complex → Invasion → PALB2–BRCA2 enhancing D-loop formation

**M:** MRE11  
**R:** RAD50  
**N:** NBS1

**Mediators (RAD52 group):**  
RAD51AP, RAD52  
RAD51B, C, D, XRCC2, XRCC3  
RAD54, RAD54B,
## SYNDROMES RELATED TO HR DEFECTS

<table>
<thead>
<tr>
<th>SYNDROMES</th>
<th>GENE</th>
<th>FUNCTION in HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nijmegen breakage syndrome (NBS)</td>
<td>NBS1, RAD50</td>
<td>resection</td>
</tr>
<tr>
<td>Ataxia telangiectasia like disease (ATLD)</td>
<td>MRE11</td>
<td>resection</td>
</tr>
<tr>
<td>Fanconi anemia (group D1)</td>
<td>BRCA2, RAD52 group*</td>
<td>RAD51 loading</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hereditary breast cancer</td>
<td>BRCA1, 2, RAD52 group**</td>
<td>various steps in recombination</td>
</tr>
<tr>
<td>Bloom syndrome (RecQ-like)</td>
<td>BLM</td>
<td>resection, resolution</td>
</tr>
<tr>
<td>Seckel syndrome</td>
<td>CtIP</td>
<td>resection</td>
</tr>
</tbody>
</table>

* RAD51C, PALB2, BRIP1
** RAD51C, D, XRCC2, PALB2, RAD50, NBS1, BRIP1
• In human cells repair of DSB is mediated by error prone NHEJ and error free HR depending on the stage of cell cycle.

• Repair of DSB requires
  core factors
  kinases, ubiquitin ligases, Dubs, SUMO ligases to induce
  posttranslational modifications of chromatin(histones) and proteins
  chromatin remodelers

• Mutations in Core factors, Kinases, Ubiquitin ligases and chromatin remodelers are linked to radiosensitive human disorders and disease
  Kinase ATM (Ataxia telangiectasia)
  Ubiquitin ligase RNF8 (Riddle syndrome)
  Chromatin remodeler CHD4 (cancer)
CELLULAR AND TISSUE RESPONSES TO DNA DAMAGE

DNA Damage Signaling

- Damage bypass
- Chromatin Remodeling
- apoptosis
- DNA repair
- Cell cycle Arrest
- Transcription responses

No/misrepair replication

Genetic alterations

DNA Damage Signaling

Genomic Instability

Epigenetic alterations

Disease cancer

Transmission of DNA damage

Acute (early) response DNA damage response (DDR)

Disease
CELLULAR AND TISSUE RESPONSES TO DNA DAMAGE

NON TARGETED EFFECTS

cell to cell signaling

Delayed genomic instability

Bystander effects

Adaptive response

Reactive oxygen driven stress response?

Disease cancer

Disease
Low dose ionizing radiation
below 200 mGy (UNSCEAR) or 100 mGy (others, EU)
dose rate 0.1 mGy min⁻¹ (UNSCEAR)

Starting point
Radiation acts primarily by inducing DNA damage in somatic and germ cells
- DNA base damage
- DNA strand breaks
- Clustered DNA damage

spontaneous DNA damage in cultured cells: 1 x 10⁻⁷ lesions/base
100 mGy: 10-20% of spontaneous oxidative damage
- increased DSB: 4 DSB

Cramers et al, Rad Res 2011
LOW DOSE IR RISK MODELS

Linear no-threshold model

- Dose response relationships
- Mechanisms

➢ Do the processes that drive carcinogenesis due to high IR dose also contribute to low dose radiation carcinogenesis?

➢ Are biological responses at low and protracted doses similar to those observed at high acute doses?

CELLULAR AND TISSUE RESPONSES TO DNA DAMAGE

DNA Damage Signaling

- Damage bypass
- Chromatin Remodeling
- apoptosis
- DNA repair
- Cell cycle Arrest
- Transcription responses

Acute (early) response
DNA damage response (DDR)

Transmission of DNA damage
Genomic Instability
Disease cancer

No/misrepair
replication

Genetic alterations
Epigenetic alterations

Disease
DNA DAMAGE SIGNALLING
PHOSPHORYLATION OF ATM and H2AX (FOCI)


SILAC (unbiased)

Light

Heavey

Radiation
Chemicals

4hr

control

Phosphopeptide enrichment

Nano LC-MS/MS

Phosphopeptide identification and quantification

PHOSPHO PROTEOMICS

No effect

Hyperphosphorylation

Hypophosphorylation

BRCA1-pSer1422

CHK1-pSer317

ATM-pSer1987

MDM2-pSer183

CTNNB1-pSer196

GSNK1A1-pSer3

SilAC Ratio H/L Normalized

Phosphopeptides
### PHOSPHOPROTEOME PROFILE

**4HRS AFTER 1Gy AND 100MGy X-RAYS**

#### ATM/ATR (>2 fold) Fold change (LOG2)

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Positions within proteins</th>
<th>Modified sequence</th>
<th>100 mGy</th>
<th>1 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atm</td>
<td>1891</td>
<td>1,1662</td>
<td>1,1604</td>
<td></td>
</tr>
<tr>
<td>Atm</td>
<td>1895</td>
<td>1,0004</td>
<td>1,0564</td>
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<tr>
<td>Atm</td>
<td>1987</td>
<td>1,3599</td>
<td>2,7644</td>
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<tr>
<td>Atm</td>
<td>3006</td>
<td>1,9786</td>
<td>2,9375</td>
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</tr>
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</table>

*S(p)1981*

Kozlov et al, JBC, 2011
<table>
<thead>
<tr>
<th>Kinase</th>
<th>cisPlatin(^1)</th>
<th>Neocarzinostatin(^2)</th>
<th>20mGy(^3)</th>
<th>500mGy(^3,4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA repair</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (weak)</td>
<td>Yes</td>
</tr>
<tr>
<td>Cell cycle / mitose</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromatin</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>RNA synthesis</td>
<td></td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Splicesome</td>
<td></td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>MAPK, PKA</td>
<td></td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Cytoskeleton/ microtubuli</td>
<td>Yes</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

1. Pines et al, MCB, 2011
2. Bensimon et al, Science sign, 2010
3. Yang et al, PlosOne, 2010
HIGH DOSE AND LOW DOSE TRANSCRIPTOMICS
LYMPHOCYTES OF 106 INDIVIDUALS

Total genes 20243

Meglicz, Mišovic, Vrieling and Giphart-Gassler, 2013
REPAIR OF DSB IN HUMAN CELLS

γH2AX foci after 2 Gy X-ray

Heterochromatin

Löbrich et al., 2005

Rothkamm et al. Proc Natl Acad Sci. 2003
SUMMARY 3 (LOW DOSE RESPONSE)

• No global threshold for DNA damage signaling (20-50mGy) ATM, H2AX, p53, CHK1/2 (Short et al, Rad Research 2005).

• Lack of information on the impact of gene defects on genome instability at low dose.

• Threshold in G2 checkpoint activation (100mGy) (Krempler et al, Cell Cycle, 2007)

• Apoptosis occurs at very low dose (2mGy) (Portess et al, Cancer Research 2007)

• Repair rates are the same at high and low dose (Suzuki et al, Rad Research 2006).

• A single DSB can activate the repair complex formation (Rodrigue et al, 2006). However, DSB repair is abrogated at 1.2 mGy (Rothkamp et al, PNAS 2003)
At present no generally agreed patterns of gene expression changes associated with exposures to different doses or dose rates.

Profound inter-individual variation in gene expression patterns.

Cellular studies on radiation and proteomics are limited and inconclusive with respect to dose or dose rates.

There are indications that low dose and low-dose rate responses differ from high dose and high dose rate responses for endpoints such as gene expression and proteomics.
CELLULAR AND TISSUE RESPONSES TO DNA DAMAGE

DNA Damage Signaling

- DNA repair
- Cell cycle Arrest
- Transcription responses
- Damaged bypass
- Chromatin Remodeling
- Apoptosis

No/misrepair replication

Genetic alterations

Genomic Instability

Disease

Transmission of DNA damage

Acute (early) response DNA damage response (DDR)

Epigenetic alterations
proto-oncogenes  tumorsuppressor genes

proto-oncogenes

dominant

1\textsuperscript{st} hit

1\textsuperscript{st} hit

2\textsuperscript{nd} hit

recessive

Deregulated function

1\textsuperscript{st} hit

2\textsuperscript{nd} hit

loss of function

hereditary forms of cancer
LOSS OF HETEROZYGOSITY (LOH)

2nd hit

Mitotic non-disjunction
Mitotic recombination
Gene conversion
Translocation
LOH IN HUMAN LYMPHOBLASTOID CELLS

A.

![Graph showing HLA-A2 loss vs. radiation dose for WIL2-NS and TK6 cells.]

B.

![Bar graph showing HLA-A2 expression with different radiation doses for TK6 and WIL2-NS cells.]

Boei et al, Rad Res 2011
MICRONUCLEI (MN) INDUCTION USING HUMAN FIBROBLASTS

**S-phase**

- **Figure 1:** Graph showing the relationship between radiation dose (mGy) and cells with MN (%). The graph includes data points for different radiation doses and shows a clear trend.

- **Figure 2:** Bar graph comparing MN per 100 BN-cells across different conditions: 0 mGy, 100 mGy (G1), 100 mGy (Cycling), 0 mGy, 100 mGy (G1), 100 mGy (Cycling). The graph displays the MN frequency in WT and Artemis cells.

- **Figure 3:** Graph illustrating MN-RET frequency as a function of dose (mGy). The graph includes linear regression lines with R² values.

- **Manning et al., Mut Res 2014**

- **Boei et al., Rad Res 2011**
MICRONUCLEI (MN) INDUCTION AND DOSE RATE

De Toledo et al, Rad Res 2006
Forment et al, Chromothripsis and cancer: causes and consequences of chromosome scattering, Nature Reviews 2012

Stephens et al, Massive chromosome rearrangements acquired a single catastrophic event during cancer development, Cell 2011

Frequency of chromothripsis in tumours (2012)

- 3% of diverse cancer cell types
- 25% of bone cancer
- 18% of neuroblastoma

Micronuclei

Casta et al, Nature 2012
Figure 2

**CHROMOTHRIPSIS AND MN**

M1

- Nocodazole
- Release

- Mitotic shake-off
- MN formation
- Selective EdU labeling of MN

G1 S-phase G2

EdU labelling

Crasta et al, Nature 2012
Figure 2

CHROMOTHRIIPSIS AND MN

M1

Nocodazole → Release
Mitotic shake-off

M2

Selective EdU labeling of MN

MN formation

Uptake of MN content

G1
S-phase EdU labelling
G2

Crasta et al, Nature 2012
Figure 2

M1
- Nocodazole
- Mitotic shake-off
- Release
- Selective EdU labeling of MN

M2
- IR
- Uptake of MN content

M3
- LOH & Chromothripsis

G1 S-phase EdU labelling G2

Crasta et al, Nature 2012
Figure 2

CHROMOTHRIPSIS AND MN

M1

Nocodazole → Mitotic shake-off → Release

Selective EdU labeling of MN

M2

IR → Uptake of MN content

M3

LOH & Chromothripsis

G1 S-phase EdU labelling G2

Crasta et al, Nature 2012

Mayak river population, unpublished results
UPTAKE OF FRAGMENTED CHROMOSOMES AFTER SECOND MITOSIS

Noco \[\text{MN formation}\] \[\text{Incubate}\]

\[\text{M1}\quad \text{M2}\]

\[\text{= Shake-off}\quad \text{= Presence of EdU}\]

Boei et al, unpublished
SUMMARY 4

- No low dose threshold for cancer related genetic damage in spite of very effective DNA damage response

- Chromosomal damage is enhanced in cells from human DDR syndromes

- Cellular responses to high and low dose are partly similar and partly specific (transcriptomics, proteomics)

- Mechanism of low dose cancer induction might involve genetic damage mediated chromothrypsis
Questions and Answers

U.S. Department of Health and Human Services
National Institutes of Health | National Cancer Institute
www.dceg.cancer.gov/RadEpiCourse
1-800-4-CANCER
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