Repair of ionizing radiation-induced DNA damage

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How does IR damage DNA?

How is IR-induced DNA damage repaired?

Practical aspects:
  - measuring DNA damage and repair

Translational aspects:
  - what has all this got to do with the clinic?
Types of DNA damage

Base damage
- Spontaneous loss of bases
- Alkylation of bases
- Oxidation of bases

UV-light induced DNA damage:
- Cyclobutane dimers
- 6,4,-photoproducts

DNA strand breaks
- Background radiation
- Medical procedures
  - X rays
  - Radiation therapy
  - Chemotherapy
Ionizing Radiation

X-rays, $\gamma$-rays, cosmic radiation/particle radiation
Energy to dislodge an electron from target molecule
Sources of ionizing radiation (IR) exposure

Here 1 Sv (Sievert) considered = 1 Gy (Gray)
1 Gy = 1 Joule energy absorbed per kg matter

**Radiation therapy:**
Typically 1.8 - 2 Gy per day; ~30 - 40 fractions for cumulative dose of 60 - 80 Gy
Higher single doses also used (brachytherapy etc)

>100 mSv increased cancer risk; <100 mSv, increased risk is less clear

Adapted from Lobrich and Jeggo, Nature Reviews Cancer (2007), 7, 861-869
**IR-induces DNA damage**

Base damage
DNA single strand breaks (SSBs)
DNA double strand breaks (DSBs):
  - 2 SSBs on opposite strands of DNA
  - 6-10 bp apart

IR-induced DSBs complex:
  - **clustered DNA damage**
    - multiple lesions within
    - one helical turn

IR-induced **strand breaks** frequently end with **non-ligatable end groups**
  - 3’-phosphates
  - 3’-phosphoglycolates
  - 5’-hydroxyl groups

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[Example of damage to the sugar-phosphate moieties induced by ionizing radiation](http://jolisfukyu.tokai-sc.jaea.go.jp/fukyu/mirai-en/2008/6_5.html)
**IR-induced DNA damage**

**Direct damage**

IR deposits energy directly in the DNA (~60%)

Damage caused by scatter electrons (red) as well as the primary radiation track (black).

**Indirect DNA damage**

IR ionizes water molecules along the radiation track, leading to the local generation of hydroxyl radicals which also damage the DNA (~40%).
Linear Energy Transfer (LET)

Low LET
- gamma radiation, X-rays
- low concentration of ionization events along the radiation track

High LET
- alpha-particles, fast neutrons
- high concentration of ionization events along the radiation track

Low LET:
- 30-40% DSB
- = complex lesions

High LET:
- >90% DSB
- = complex lesions

Same number of ionization events = same dose
High LET more damaging
- damage more concentrated

http://www.rerf.jp/radfx/basicknoe/radcell.htm
IR induces base damage (stars)

And single strand breaks (SSBs) that often have non-ligatable end groups (3’ phosphate/phosphoglycolate; 5’ -OH) (red)

2 SSBs on opposite strands ~6-10bp apart results in a DSB

1 Gy IR produces thousands of base lesions, hundreds of DNA single strand breaks but only tens of DSB

DSBs are the most cytotoxic
Outline

• How does IR damage DNA?

• How is IR-induced DNA damage repaired?

Repair of
  Base damage
  Single strand breaks (SSBs)
  DNA double strand breaks (DSBs)
IR induces base oxidation, base damage and loss of bases

IR induced base damage repaired by the **base excision repair pathway**

Damaged base removed by **DNA glycosylase** (e.g. OGG1 removes 8-oxo guanine)

**AP endonuclease** (APE1) creates nick (singe strand break) in the DNA backbone at the abasic site

**DNA polymerase beta** adds new nucleotide

**DNA ligase I/III** seals DNA backbone

- Multiple DNA glycosylases
- Two pathways depending on type of base damage and which glycosylase is involved
- Other proteins involved in BER include **XRCC1**, Flap endonuclease (**FEN1**)
Short Patch and Long Patch Base Excision Repair

DNA pol-beta, XRCC1/DNA ligase III and Accounts for at least 80% of BER

Requires DNA pol beta, FEN1, and DNA ligase I (RPA, PCNA, RFC, WRN). Occurs when DNA pol-beta cannot remove the 5’-dRP blocking group.

Repair of IR-induced DNA

Single Strand Breaks (SSBs)

Variation of Base Excision Repair

Polynucleotide kinase/phosphatase (PNKP):
  removes 3’phosphate groups/adds 5’-hydroxyl groups

Poly-ADP ribose polymerase (PARP 1):
  binds DNA SSBs
  uses NAD⁺ as substrate to catalyze formation of long, branched polymers of poly-ADP ribose

Tyrosine DNA phosphodiesterase 1 (TDP1):
  converts 3’-phosphoglycolates to 3’-phosphatase that are removed by PNKP

XRCC1: scaffolding protein (interacts with DNA ligase I/III/PNKP/PARP1)

DNA ligase I/III: seals the break
Repair of IR-induced DNA Single Strand Breaks (SSBs)

Involves BER core machinery, plus poly ADP ribose polymerase (PARP-1), polynucleotide kinase (PNK), tyrosyl DNA phosphodiesterase (Tdp1)

Almeida and Sobol DNA Repair (2007)
Repair of IR-induced DNA double strand breaks (DSBs)

2 main DSB Repair pathways:

**Non-homologous end joining (NHEJ)**
- Ku, DNA-PKcs, XRCC4, Artemis, XLF, PAXX, DNA ligase IV
- (also PNKP and others)

**Homology directed repair (HDR/HRR)**
- Rad51, Rad52, XRCC2, XRCC3, BRCA2 + others

Also **Alternative end-joining** (PARP1, XRCC1, DNA ligase I/III)

**DSB signaling response**
- Ataxia telangiectasia mutated (ATM) (also ATM related, ATR) - mediated phosphorylation cascades and phosphorylation of **histone H2AX**,
  mediating cell cycle checkpoints and other cellular endpoints
Cellular response to IR-induced DSBs

Non-Homologous End Joining (NHEJ)
>80% DSBs
G1, G2

Homologous Recombination Repair (HRR)
<20% DSBs
S,G2

Adapted from Wang and Lees-Miller, IJRBB, 2013
Model for NHEJ showing role of DNA-PKcs autophosphorylation

1. DSB detection and tethering:
   - Ku, DNA-PKcs

2. End Processing:
   - Artemis, PNKP, Pol mu/pol lambda
   - APLF, Mre11? Tdp1? WRN1?

3. Ligation:
   - XRCC4, DNA ligase IV, XLF

- Dissociation of autophosphorylated DNA-PKcs
Non-homologous end joining (NHEJ)

Active G1, G2, possibly S
Major pathway for repair of IR-induced DSBs in mammalian cells

Detection of the DSB
- Ku70/80 heterodimer
- DNA-dependent protein kinase catalytic subunit (DNA-PKcs): recruited to DNA-bound Ku, moves Ku inwards, protects the DSB ends

Processing of non-ligatable DNA termini
- Polynucleotide kinase/phosphatase (PNKP): removes 3’-phosphates/adds 5’-phosphates
- Aprataxin/PNKP related factor (APLF): exonuclease: removes damaged ends?
- Artemis: exo/endonuclease interacts with DNA-PKcs
- DNA polymerases mu and lambda: filling in of DNA gaps?

DNA ligation
- DNA ligase IV: exists in complex with XRCC4, XLF and possibly PAXX
  (XRCC4: X-ray cross complementing group 4; XLF: XRCC4-like factor; PAXX- paralog of XRCC4 and XLF).

DNA-PKcs kinase autophosphorylation required for regulating end processing:
- DNA-PKcs binds, autophosphorylates, released, to regulate access of processing factors to DSB
Model for NHEJ emphasizing the central role of Ku

Adapted from Radhakrishnan and Lees-Miller, DNA Repair, 2014
Cellular response to IR-induced DSBs

Non-Homologous End Joining (NHEJ)
- >80% DSBs
- G1, G2

Homologous Recombination Repair (HRR)
- <20% DSBs
- S, G2

Adapted from Wang and Lees-Miller, IJRBB, 2013
Alt-NHEJ:

Requires PARP-1, XRCC1, DNA ligase III.

Shown to function in cells that are unable to carry out classical-NHEJ

Highly error prone, associated with large deletions/translocations
Cellular response to IR-induced DSBs

Non-Homologous End Joining (NHEJ)
>80% DSBs
G1, G2

Homologous Recombination Repair (HRR)
<20% DSBs
S/G2

Adapted from Wang and Lees-Miller, IJRBB, 2013
Repair of IR-induced DSBs by Homologous Recombination Repair (HRR)

Active only in S and G2

Requires undamaged sister chromatid as template for accurate repair

Initiated by binding of Mre11, Rad50, Nbs1 (MRN) complex to DSB ends

Recruitment of CtIP, DNase2, Exonuclease:
   5’-3’ resection of ends of DSB to yield long regions of ssDNA with 3’-overhang:
   only in S and G2

RPA binds ssDNA

BRCA2 required to displace RPA and replace it with Rad51

Rad51 forms long nucleoprotein filaments on ssDNA to initiate strand invasion

Subsequent steps not well understood, involve branch migration, new DNA synthesis using undamaged sister chromatid as template

Final ligation of DNA ends
Model for repair of DSBs by Homologous Recombination Repair

MRN complex (Mre11, Rad50, Nbs1) binds to DSB

MRN-dependent 5’-3’ resection to generate long ssDNA with 3’ end (also requires other nucleases- CtIP, DNase2, Exo1)

RPA binds to ssDNA

BRCA2 and other proteins promote displacement of RPA and loading of Rad51 to form nucleoprotein filament that facilitates strand invasion.

Repair synthesis: DNA pol eta?

Branch migration: WRN, BLM, p53, Rad54, BLAP75, MSH2/ MSH6??

Flap removal: ERCC1/XPF??

Synthesis/ligation: pol delta/epsilon, PCNA, DNA ligase I ???

dsDNA with DSB

Undamaged sister chromatid

5’-3’ resection of ends to produce long ss DNA with 3’-OH

Strand invasion to produce heteroduplex (D loop)

DNA synthesis using sister chromatid as template

Branch migration of Holliday junction

3’-OH can anneal to opposite end of original DSB

Repair synthesis/gene conversion

**Review: Helleday et al, 2007**
Cellular response to IR-induced DSBs

Non-Homologous End Joining (NHEJ)
>80% DSBs
G1, G2

Homologous Recombination Repair (HRR)
<20% DSBs
S, G2

Adapted from Wang and Lees-Miller, IJRBB, 2013
The phosphatidyl inositol 3 kinase-like protein kinases (PIKKs)

- DNA-dependent protein kinase catalytic subunit (DNA-PKcs)
- Ataxia-telangiectasia mutated (ATM)
- ATM-, Rad3-related (ATR)

Serine/threonine protein kinases
Share a similar architecture:
- N-terminal helical domain (HEAT repeats)
- FAT domain
- kinase domain
- FATC domain

Inhibited by wortmannin
Phosphorylate substrates preferentially on Ser-Gln or Thr-Gln (SQ/TQ motifs)
Ataxia Telangiectasia Mutated (ATM)

**Ataxia-telangiectasia (A-T):**
Autosomal recessive; compound heterozygotes
Incidence 1 in ~ 40,000 to 1 in 100,000 live births
Characterized by neurodegeneration, progressive loss of
neuromuscular control, ataxia, telangiectasia,
immune deficiencies, cancer predisposition (lymphoma),
radiation sensitivity
Over 400 mutations identified to date
Mutations occur throughout the gene and are usually truncation or
splicing; about 10% are mis-sense

**ATM heterozygotes:** increased risk for breast cancer

**Cell Lines lacking ATM:**
Highly radiosensitive
Radiation resistant DNA synthesis,
Cell Cycle checkpoint defects G1/S, intra S, G2/M
Chromosomal breakage
Genomic instability
Activation of ATM

IR induces DSB

MRN complex (Mre11, Rad50, Nbs1) binds DSB and recruits ATM (inactive form as a dimer)

ATM undergoes dissociation from dimer to monomer and autophosphorylation on S1981 and other sites

Phosphospecific antibody to phosphorylated-serine 1981 frequently used as marker of ATM activation

DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation.
ATM-dependent signaling ~ 2004

Kurz and Lees-Miller, DNA Repair, 2004
ATM and ATR Substrate Analysis Reveals Extensive Protein Networks Responsive to DNA Damage

Shuhei Matsuoka,1 Bryan A. Ballif,2 Agata Smogorzewska,3,4† E. Robert McDonald III,2† Kristen E. Hurov,1† Ji Luo,3† Corey E. Bakalarski,2 Zhenming Zhao,4 Nicole Solimini,3 Yaniv Lerenthal,4 Yosef Shiloh,4 Steven P. Gygi,2‡ Stephen J. Elledge2‡

Cellular responses to DNA damage are mediated by a number of protein kinases, including ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3-related). The outlines of the signal transduction portion of this pathway are known, but little is known about the physiological scope of the DNA damage response (DDR). We performed a large-scale proteomic analysis of proteins phosphorylated in response to DNA damage on consensus sites recognized by ATM and ATR and identified more than 900 regulated phosphorylation sites encompassing over 700 proteins. Functional analysis of a subset of this data set indicated that this list is highly enriched for proteins involved in the DDR. This set of proteins is highly interconnected, and we identified a large number of protein modules and networks not previously linked to the DDR. This database paints a much broader landscape for the DDR than was previously appreciated and opens new avenues of investigation into the responses to DNA damage in mammals.

Shuhei Matsuoka, et al.
Science 316, 1160 (2007);
IR-induced foci (IRIF) and γ-H2AX

Created by PIKK-dependent phosphorylation of histone H2AX

**H2AX:**

Variant of histone H2A

Represents 5-25% of total H2A in mammalian cells (100% yeast H2A is H2AX)

About 1 in 5 nucleosomes contains H2AX instead of H2A

Contains C-terminal “tail” (13 amino acids) with SQ (S139 in humans)

Phosphorylation of H2AX on S139 (primarily by ATM) creates gamma-H2AX (γ-H2AX)

\[
\begin{array}{cccccccc}
\text{Residue number} & 80 & 90 & 100 & 110 & 120 & 130 & 140 \\
\text{HUMAN 1 (P)} & \text{PRHLQ|AIRNDEELNL|GGVTV|QAVLP|KKE|SHHKAKGK}^{*} \\
\text{HUMAN 1 (L)} & \text{PRHLQ|AIRNDEELNL|G|VTV|QAVLP|K|SHHKAKGK}^{*} \\
\text{HUMAN X} & \text{PRHLQ|AIRNDEELNL|GGVTV|QAVLP|K|TSATVFAKSGGKATGASQEY}^{*} \\
\text{MOUSE X} & \text{PRHLQ|AIRNDEELNL|GGVTV|QAVLP|KSSATVFA|PAVGK|KASQEY}^{*}
\end{array}
\]

γ-H2AX detected using a phospho-specific antibody to phosphorylated S139: western blot, immunofluorescence, immunohistochemistry
IR-induced foci (IRIF) and γ-H2AX

1 Gray (Gy) of IR induces about 30-40 DSB per cell

Phospho specific antibody to serine 139 phosphorylated H2AX (termed γ-H2AX) reveals 30-40 IR-induced foci (IRIF) per Gy

Widely assumed that 1 H2AX foci = 1 DSB

Each foci is ~ 0.5 µm diameter and represents thousands of molecules of phosphorylated H2AX surrounding the DSB and extending over several Mb of chromatin either side of the DSB (but not at the break)

Immunofluorescence of human cells: unirradiated (left) or 30 min after IR (right)

ChIP from defined DSB in human cells

No γ-H2AX at the DSB;
γ-H2AX foci radiate out from the DSB;
ATM and Nbs1 are at DSB and at distal sites
(Berkovich et al, Nature Cell Biology, 2007)
**Kinetics of γ-H2AX foci**

**Transient**
First observed ~ 15 minutes  
Peak ~1 hour,  
Decrease by 2-4 hours  
In normal cells, baseline levels 12-24 hours**

**Persistent foci = unrepaired DSBs**
Persistent foci in XLF, DNA-PKcs  
or Ligase IV null cells

(∗∗caveat: gamma-H2AX indirect measure of DSBs: measuring histone phosphorylation. Protein phosphatases also remove γ-H2AX.)

**Resolution of foci is biphasic**
Fast response: 0-2 hours: repair by NHEJ

Slow response: 2-24 hours  
ATM-dependent repair of heterochromatic DSBs/complex DSBs

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Riballo et al, *Mol Cell* 2004

Yu et al, *DNA Repair*, 2008

XLF-deficient

Normal cells

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Multiple proteins assemble at RIF Requires protein phosphorylation and ubiquitination

- MRN binds DSB; activates ATM

- ATM phosphorylates H2AX to create γ-H2AX

- MDC1 binds γ-H2AX directly via BRCT domain

- ATM phosphorylates MDC1, creating binding site for RNF8

- ATM phosphorylates additional H2AX creating more MDC1 binding sites (amplification of γ-H2AX signal)

- RNF8/Ubc13 ubiquitinate H2A/H2AX creating binding sites for RNF168

- RNF168/Ubc13 create poly-Ub chains that recruit 53BP1 and the Rap80, abraxas, BRCA1 complex

* RNF8 and RNF168 = E3 Ubiquitin Ligases

Van Attikum and Gasser TCB 2009
Outline

• How does IR damage DNA?

• How is IR-induced DNA damage repaired?

• Practical aspects:
  • measuring DNA damage and repair

• Translational aspects:
  • what has all this got to do with the clinic?
Measuring DSBs in cells

Kinetics of γ-H2AX foci with time as indication of repair of DSBs (indirect but sensitive and robust)


Simonsson et al, Rad. Oncol. 2008
Methods for the direct measurements of DNA damage in cells

**The Comet Assay**
Neutral: DSB
Alkaline: SSB and DSB

**Pulsed Field Electrophoresis:**
Fraction of activity remaining (FAR assay)

Repair after exposure to 40 Gy X-rays

NHEJ-defective cell line

Normal cell line
• How does IR damage DNA?

• How is IR-induced DNA damage repaired?

• Practical aspects:
  • measuring DNA damage and repair

• Translational aspects:
  • what has all this got to do with the clinic?
Loss or inactivation of genes/proteins involved in DNA single strand or DNA double strand break repair pathways leads to increased cellular radiation sensitivity

Clonogenic survival assay/colony formation assay

Example:

Mouse embryonic fibroblasts with knock out of **XRCC1** or DNA polymerase beta (BER/SSBR) are modestly radiation sensitive

Horton et al, Cell Research 2008
Cells lacking ATM or DNA-PKcs are radiation sensitive

Radiation sensitivity of A-T cells (lack ATM) compared to normal human fibroblasts cell lines


Radiation sensitivity of M059J cells that lack DNA-PKcs compared to MO59K cells with normal levels of DNA-PKcs

Merry and Lees-Miller, unpublished
Inactivation/deletion of DNA damage response proteins induces radiation sensitivity

<table>
<thead>
<tr>
<th>Mammalian mutant</th>
<th>Animal viability</th>
<th>Day 3.5 embryo/blastocysts</th>
<th>IR sensitivity ES cells</th>
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Brugmans et al, Mutation Research, 2007

**Ataxia telangiectasia-mutated (ATM) gene polymorphisms and acute normal tissue injuries in cancer patients after radiation therapy: a systematic review and meta-analysis.**

**PURPOSE:** Studies of the association between ataxia telangiectasia-mutated (ATM) gene polymorphisms and acute radiation injuries are often small in sample size, and the results are inconsistent. We conducted the first meta-analysis to provide a systematic review of published findings.

**METHODS AND MATERIALS:** Publications were identified by searching PubMed up to April 25, 2014. Primary meta-analysis was performed for all acute radiation injuries, and subgroup meta-analyses were based on clinical endpoint. The influence of sample size and radiation injury incidence on genetic effects was estimated in sensitivity analyses. Power calculations were also conducted.

**RESULTS:** The meta-analysis was conducted on the ATM polymorphism rs1801516, including 5 studies with 1588 participants. For all studies, the cut-off for differentiating cases from controls was grade 2 acute radiation injuries. The primary meta-analysis showed a significant association with overall acute radiation injuries (allelic model: odds ratio = 1.33, 95% confidence interval: 1.04-1.71). Subgroup analyses detected an association between the rs1801516 polymorphism and a significant increase in urinary and lower gastrointestinal injuries and an increase in skin injury that was not statistically significant. There was no between-study heterogeneity in any meta-analyses. In the sensitivity analyses, small studies did not show larger effects than large studies. In addition, studies with high incidence of acute radiation injuries showed larger effects than studies with low incidence. Power calculations revealed that the statistical power of the primary meta-analysis was borderline, whereas there was adequate power for the subgroup analysis of studies with high incidence of acute radiation injuries.

**CONCLUSIONS:** Our meta-analysis showed a consistency of the results from the overall and subgroup analyses. We also showed that the genetic effect of the rs1801516 polymorphism on acute radiation injuries was dependent on the incidence of the injury. **These support the evidence of an association between the rs1801516 polymorphism in ATM and acute radiation injuries, encouraging further research of this topic.**
Inhibition of ATM or DNA-PKcs radiosensitizes cells

DNA-PKcs, ATM and ATR are protein kinases

Small molecule inhibitors of DNA-PKcs and ATM radiosensitize cell lines

ATM inhibitor: KU55933
DNA-PKcs inhibitor: NU7441, NU7026
ATR inhibitor: AZ20

Hickson et al, Cancer Research, 2004
Zhao et al, Cancer Research, 2006

Potential for use in patients? Therapeutic index?
**DNA damage response inhibitors in clinical trials**

<table>
<thead>
<tr>
<th>Target</th>
<th>Inhibitor</th>
<th>Mono- or combination therapy / clinical study stage</th>
<th>Clinical trial identifier / reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>KU-55833</td>
<td>IR, etoposide, doxorubicin, camptothecin, in preclinical testing</td>
<td>[25, 58]</td>
</tr>
<tr>
<td></td>
<td>KU-60019</td>
<td>IR in preclinical testing using glioma cells</td>
<td>[27]</td>
</tr>
<tr>
<td>ATR</td>
<td>NU-6027</td>
<td>Hydroxyurea, cisplatin, temozolomide, rucaparib in preclinical testing</td>
<td>[29]</td>
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<tr>
<td></td>
<td>VE-821</td>
<td>Cisplatin in breast and ovarian cell lines</td>
<td>[31, 90]</td>
</tr>
<tr>
<td></td>
<td>ETP-85464</td>
<td>IR, gemcitabine in pancreatic cancer cells in preclinical testing</td>
<td>[32]</td>
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<tr>
<td>DNA-PKcs</td>
<td>NU-7441</td>
<td>IR, etoposide in preclinical testing of cancer cell lines and tumour xenografts</td>
<td>[44, 97]</td>
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<tr>
<td></td>
<td>NU-7026</td>
<td>IR and combined with AG14381 (PARP1) in preclinical testing</td>
<td>[43]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anthracyclines, mitoxantrone, etoposide in preclinical testing using leukaemia cells</td>
<td>[99]</td>
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<tr>
<td>DNA-PKcs/PI3K</td>
<td>KU-60648</td>
<td>Etoposide, doxorubicin in preclinical testing</td>
<td>[45]</td>
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<tr>
<td>DNA-PKcs/mTOR</td>
<td>CC-115</td>
<td>Single agent in Phase I safety and tolerability study (recruiting)</td>
<td>NCT01263625</td>
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<tr>
<td>PI3K/mTOR/PIKK</td>
<td>NVP-DEZ235</td>
<td>Single agent in several clinical trials</td>
<td>[34, 36]</td>
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<tr>
<td>CHK1/CHK2</td>
<td>UCN-01</td>
<td>Single agent in Phase II for relapsed T-cell lymphoma (completed)</td>
<td>NCT000092017</td>
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<td></td>
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<td>Single agent in Phase II for metastatic melanoma (completed)</td>
<td>NCT0072189</td>
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<td></td>
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<td>Five fluorouracil in Phase II for metastatic pancreatic cancer (completed)</td>
<td>NCT00045747</td>
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<td>Topotecan in Phase II for various forms of ovarian cancer (completed)</td>
<td>NCT03072267</td>
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<td></td>
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<td>Topotecan in Phase II for small cell lung cancer (completed)</td>
<td>NCT00086956</td>
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<tr>
<td></td>
<td></td>
<td>Olaparib in pre-clinical testing for multiple mammary tumour types</td>
<td>[99]</td>
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<tr>
<td>CDC25</td>
<td>MK-1775</td>
<td>Carboplatin in Phase III for epithelial ovarian cancer</td>
<td>NCT01168895</td>
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<tr>
<td>MRE11</td>
<td>BRC-083864</td>
<td>Single agent in preclinical testing using pancreatic and prostate cancer cells</td>
<td>[42]</td>
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<tr>
<td></td>
<td></td>
<td>Single agent or with olaparib (PARP1) in preclinical testing using BRCA1-deficient cells</td>
<td>[47]</td>
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<tr>
<td>RPA</td>
<td>MC313E</td>
<td>Single agent or with cisplatin in preclinical testing</td>
<td>[43]</td>
</tr>
<tr>
<td>RAD51</td>
<td>R02</td>
<td>IR, mitomycin C, cisplatin in preclinical testing</td>
<td>[90]</td>
</tr>
<tr>
<td></td>
<td>R8-1</td>
<td>Mitomycin C in preclinical testing</td>
<td>[50]</td>
</tr>
</tbody>
</table>

ADP = adenosine diphosphate, ATM = ataxia telangiectasia mutated protein, ATR = ATM- and Rad3-related, CHK = checkpoint kinase, DNA = deoxyribonucleic acid, DNA-PK = DNA-dependent protein kinase; DNA-PKcs = DNA-PK catalytic subunit; IR = ionizing radiation; mTOR = mammalian target of rapamycin; PARP = poly(ADP-ribose); PARP = PAR polymerase; PARPI = PARP inhibitor; PI3K = phosphatidylinositol-3-kinase; PIKK = PI3K-related kinase; RPA = replication protein A.
DNA damage response inhibitors in clinical trials

PARP inhibitors

PARP: Poly-ADP Ribose Polymerase

Multiple PARPs in cell, PARP1, PARP2 and PARP3 implicated in DNA damage response

PARP1

- Binds DNA single strand breaks (SSBs)
- Uses NAD$^+$ to synthesize long chains of poly-ADP ribose
- Recruits DNA repair proteins to sites of DNA damage/removes histones

- Involved in BER, SSBR, and possibly in cellular responses to replication fork collapse and HRRR
PARP: Poly ADP ribose polymerase

PARP binds to DNA SSBs

Uses NAD\(^+\) as substrate to create long polymers of poly-ADP ribose (PAR)

PAR:
Recruits other proteins (e.g. XRCC1) to the DNA break

Removes histones from the chromatin?

Removed by PARG (poly-ADP ribose glycohydrazase)

Facilitates recruitment of DNA repair enzymes (e.g. XRCC1)

PARP inhibitors kill BRCA1/BRCAl2-deficient cancer cells

Patients with germline BRCA1/BRCA2 mutation have one functional copy of BRCA1/2; still able to repair DSBs by HRR

Tumours lose the functional copy of BRCA1/2; unable to carry out HHR sensitive to PARP inhibitors

“Synthetic lethality”

2009: Results of phase 1 clinical trial of the PARP inhibitor olaparib in BRCA-deficient breast, ovarian and prostate cancers
Dec 2014: FDA approves the first PARP inhibitor (olaparib/Lynparza) for the treatment of ovarian cancer with defective BRCA genes
<table>
<thead>
<tr>
<th>Rank</th>
<th>Status</th>
<th>Study</th>
<th>Conditions</th>
<th>Interventions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Recruiting</td>
<td>PARP inhibitor BMN-673 and Temozolomide or Irinotecan Hydrochloride in Treating Patients With Locally Advanced or Metastatic Solid Tumors</td>
<td>Metastatic Cancer; Unspecified Adult Solid Tumor</td>
<td>Drug: PARP inhibitor BMN-673; Drug: temozolomide; Drug: irinotecan hydrochloride; Other: pharmacological study; Other: laboratory biomarker analysis</td>
</tr>
<tr>
<td>2</td>
<td>Not yet recruiting</td>
<td>PARP inhibitor BMN-673 in Treating Patients With BRCA1 and BRCA2 Wild-Type, Metastatic or Recurrent, Triple-Negative or HER2-Negative Breast Cancer</td>
<td>Estrogen Receptor Negative; HER2/Neu Negative; Male Breast Carcinoma; Progesterone Receptor Negative; Metastatic Breast Carcinoma; Stage IV Breast Cancer; Triple-Negative Breast Cancer</td>
<td>Drug: PARP inhibitor BMN-673; Other: Laboratory Biomarker Analysis</td>
</tr>
</tbody>
</table>
IR induced base damage and DNA Singe strand breaks SSBs.

2 SSBS on opposite sides of DNA creates a DSB: most cytotoxic form of IR-induced lesion

SSB/DSB ends frequently have non-ligatable termini (3’-phosphate, 5’-hydroxyl)

Main pathway for repair of IR-induced DSBs in human cell = NHEJ

Non-homologous end joining (NHEJ)
initiated by binding of the Ku70/80 heterodimer
active throughout the cell cycle
error prone: loss of nucleotides from DNA ends

Alt-NHEJ
not much known, requires PARP, XRCC1, DNA ligase III,
error prone, associated with loss of DNA

Homologous recombination repair (HRR) active only in S and G2
initiated by binding of MRN and resection to create long ssDNA overhangs
accurate, required sister chromatid as template
If NHEJ and HRR are both active in G2, what determines whether a break is detected by Ku and repaired by NHEJ versus bound by MRN, resected and repaired by HRR?

ATM is also activated by MRN (but this does not require its nuclease activity):

what determines whether a break is detected by Ku and repaired by NHEJ or bound by MRN to activate ATM (occurs throughout the cell cycle)?

Learning some things about the mechanism of NHEJ, but very little known about HRR, especially later steps.
Cells lacking DNA-PKcs, ATM and other DDR proteins are highly sensitive to IR and other DSB inducing agents.

Small molecule inhibitors of the protein kinase activity of DNA-PKcs (NU7441) and ATM (KU55933) inhibit DSB repair in cell lines.

Can they be used to radiosensitize tumours? Therapeutic index?

PARP inhibitors initially developed as radiosensitizers but shown to kill BRCA-deficient cells by “synthetic lethality”

Can other repair proteins be targeted to enhance cancer cell killing?

ATM deficient cancer cells are also sensitive to PARP inhibitors

Williamson et al, Molecular Cancer Therapeutics, 2010
Williamson et al, EMBO Molecular Medicine, 2012
Kubota et al, Cell Cycle, 2014

PNKP inhibitors radiosensitize cells

Freschauf et al, Cancer Research, 2010


Thoms and Bristow 2010. Seminars Rad Onc. 20, 217-222
Thank you!
Questions?