DNA Damage Repair Pathway and Tumorigenesis & Therapy

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normal tissue

? 
cancer

Sensitive
Resistant
Epithelial tumor development

Loss of p53

- Loss of Terc
- Loss of Brca2
- Loss of Rb
- Loss of Cdc4
- p53 mutants
- Others?

Increase in genomic instability
How does DNA Damage Repair Pathway Affect Genomic Stability?

How does DNA Damage Repair Pathway Affect Tumor Therapy?
Diseases related with DNA double strand break repair pathways

SCID: Severe Combine Immune Deficiency
- DNA-PK-SCID
- Artemis-RS-SCID

Cancer Prone: BRCA1/2 Familial breast cancer

Neurodegeneration

NHEJ mediated VDJ rearrangement

Contribution of known genes to familial aggregation of breast cancer

Other genes familial risk factors
- BRCA1
- BRCA2
- TP53
- PTEN
- ATM
- CHEK2
- BRIP1
- PALB2
- 79 common SNPs
The DNA double strand break repair pathways

Non-homologous end-joining  Homologous recombination

G1 phase  S/G2 phase

Damage Recognition

DNA end processing

Strand invasion  DNA synthesis & resolution

Blunt DNA end  Over-hanged DNA end

DNA Ligation
How DDR factors are regulated precisely?

HR and NHEJ repair pathway choice

Identify new DDR factors

Cell Reports 2017
Molecular Cell 2013
Cancer Research 2013
Journal Biol Chem 2013
PARPi (Niraparib) treatment resulted in significantly longer progression-free survival than placebo in the HRD-positive Recurrent Ovarian Cancer.
BRCA Mutated Cancer → SSB → PARPi → BRCA- → Kill Cancer → BRCA+ → Resistance

?
BRCA1 wt

ATM

RING BRCA1 BRCT

53BP1

PTIP RIF1

DSB

Resection

HR Accurate DNA repair

BRCA1 deficient

ATM

53BP1

PTIP RIF1

DSB

Resection

NHEJ Error-prone DNA repair
Artemis nuclease activity is crucial for PARPi sensitivity of BRCA deficient cells.
PARP-mediated SSB repair

DSB formation

BRCA1/2 mediated HR

Rad51

Repair breaks and cell survival

Loss of 53bp1 and its effectors
Partially restored HR

BRCA1/2 deficient cells
Error prone NHEJ
Cell death

Reversion of BRCA1/2 mutations
Restored HR activity

53bp1

PTIP

RIF1

Artemis

CtIP

Rev7

RPA
Acetylation of 53BP1 regulates the balance of HR and NHEJ and decides the resistance to PARPi.
**S phase**

**Homologous recombination**

- DSB
- HR

- MRN
- Resection
- BLM
cTIP
BRCA1
BLM
- Strand invasion
- Rad51
- DNA synthesis
- Ligation, branch migration, Holliday junction resolution

**G1 phase**

**Non-homologous end-joining**

- DSB
- NHEJ

- DNA-PKcs
- Ku80
- Ku70
- DNA-PKcs
- Artemis
- XRCC4/Ligase IV
- DNA-PKcs
- Ligation
Background

**MRE11: Ataxia-Telangiectasia-like disorder (ATLD)**

1. Neurodegeneration
2. Ataxia
3. Normomorph
4. Cancer/Genomic instability

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disease</th>
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<tr>
<td>ATM</td>
<td>Ataxia-Telangiectasia (AT)</td>
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<tr>
<td>MRE11</td>
<td>Ataxia-Telangiectasia-like disorder (ATLD)</td>
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<td>NBS1</td>
<td>Nijmegen breakage syndrome (NBS)</td>
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<tr>
<td>RAD50</td>
<td>NBS-like disorder (NBSLD)</td>
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MRN complex (MRE11/RAD50/NBS1)

MRE11 nuclease forms a trimeric complex (MRN) with RAD50 and NBS1

Homologous recombination

DNA double-strand break

Initiation of DNA resection

Long-range DNA resection

RAD51 filament formation

Strand invasion

DNA synthesis

How is MRN tightly regulated to avoid inefficient repair or unspecific resection?
C1QBP modulates DNA damage response by regulating MRE11

Molecular Cell, 2019
<table>
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<tr>
<th>Protein</th>
<th># unique peptides</th>
<th>Coverage(%)</th>
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**MRE11 purification**

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**RAD50 purification**

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**C1QBP purification**

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**NBS1 purification**

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C1QBP protein:
• First identify C1q interaction protein
• Critical for ARF induced apoptosis
• Important for mitochondrial translation
• ATM substrate

C1qbhp-null mice:
• Embryonic lethality with a severe developmental defect
• Impaired mitochondrial protein synthesis
• Immunodeficiency
C1QBP interacts with MRE11 and RAD50 in vivo
C1QBP interacts with MRE11 and RAD50 in vitro
MRN complex vs MRC complex
Sucrose gradient fraction indicated that a substantial portion of MRE11/Rad50 co-sediments with C1QBP

WT

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C1QBP KO

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Charts show relative protein abundance across different fractions for WT and C1QBP KO conditions.
C1QBP forms MRC complex with MRE11 and RAD50

Exclusive complex?

MRC complex competing with MRN complex
C1QBP inhibits MRE11 exonuclease activity
C1QBP disrupted the binding ability of MRE11/Rad50 with dsDNA and MRE11 exonuclease activity.
C1QBP could disrupt the existed MRE11/Rad50/DNA complex
C1QBP interacts with GAR domain of MRE11

GAR motif: Glycine Arginine Rich motif
R572Q and R576Q dramatically reduced the interaction between MRE11 and C1QBP.
Loss of GAR motif totally disrupts the interaction between MRE11 and C1QBP

MST (microscale thermophoresis)
DNA damage-induced MRE11 phosphorylation dismisses MRC complex
C1QBP translocated to nuclear upon DNA damage

G
NT
C1QBP
28%
23%
28%

CPT
10 μM/7 h
C1QBP
39%
38%
44%

0 10 20 30 40 min
C1QBP-GFP
C1QBP stabilizes MRE11 and RAD50 in vivo

C1QBP prevents MRE11 and RAD50 degradation
**C1QBP prevents MRE11 to associate with chromatin**

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<thead>
<tr>
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<td>Flag(C1QBP)</td>
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<td>H3</td>
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**Diagram:**
- RAD50
- MRE11
- C1QBP
- GAPDH
- H3

**Figure:**
- RAD50
- MRE11
- C1QBP
- GAPDH
- H3
Loss of C1QBP causes retarded recruitment of MRE11 to damaged chromatin.
Loss of C1QBP causes DNA end resection defects
Loss of C1QBP impair DNA repair capability

% cells >5 γH2AX foci

Time after IR(3Gy) treatment

C1QBP WT
C1QBP KO

CPT

C1QBP

WT
KO

DMSO
CPT

Tail Length

WT
KO

****
C1QBP is required for maintaining chromosome stability
Positive correlation between C1QBP and MRE11
C1QBP knockout cells displayed hypersensitivity to IR/CPT/Olaparib
Higher level of C1QBP are associated with poor prognosis
C1QBP is a potential therapeutic target for cancer treatment
Brushes: MRE11/RAD50
Container: C1QBP
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Thank You for Your Attention!