DNA Repair and Checkpoints

Cellular and Molecular Biology of Cancer
PATH G4500-001

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Goal

1. Recognize the connection between DNA repair and Cancer biology
2. Framework of DNA repair
3. Connection of DNA damage response and cell cycle checkpoints
4. Common assays for DNA repair function and genomic instability

* Some key references for additional reading are included
Overview

1. DNA damage and Cancer
2. Types and Sources of DNA Damages
3. DNA repair pathways
4. DNA damage responses
5. Cell Cycle Checkpoints
6. Special cases
   1. Development DNA breaks - Lymphocyte, Meiosis, neuronal function etc.
   2. Oncogene – how do they induce instability
   3. Telomere, mitochondria and rDNA clusters
   4. Target DNA repair for cancer therapy
1. DNA damage and Cancer

1.1. Cancer is a genetic disease – change of DNA

1.2. Types of genetic alterations are cancer type and development origin dependent
- gross rearrangement, point mutations, in between
- development origin, environment factors,……

1.3. DNA damages as the basis of conventional chemotherapy
- Selectivity (cancer vs normal) \( \simeq \) proliferation index
- Vulnerability – lack of redundant checkpoints
1.1 Cancer is a “genetic” disease

Normal → Pre-Cancer → Cancer → Residual → Relapse

Oncogene↑ Tumor Suppressor↓ "Replication Stress" "DNA damages"

"mutations" “chromosomal changes”

Bartkova et al. 2005 Nature 434, 864-870
1.2 Type of DNA lesions

Aneuploidy

Deletion

Amplification

Translocation-(NOT) trans-splicing

Mutation

Cytogenetics

1960’ ~

CGH

1990’ ~

In frame

small

RNA-seq

Exon or WG seq

2000’s~

### 1.2. Cancer type specificity

#### Translocations (inter and intra)

<table>
<thead>
<tr>
<th>Trisome chr 21</th>
<th>Down Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;14) c-Myc; IgH</td>
<td>Burkitt's lymphomas</td>
</tr>
<tr>
<td>t(11;14) CyclinD1; IgH</td>
<td>Mantle cell lymphomas</td>
</tr>
<tr>
<td>t(14;18) IgH;BCL2</td>
<td>Follicular Lymphomas</td>
</tr>
<tr>
<td>t(3;14) BCL6;IgH</td>
<td>Diffuse Large B cell lymphomas</td>
</tr>
<tr>
<td>t(1;14) TAL1; TCRα/δ</td>
<td>T-ALL</td>
</tr>
</tbody>
</table>

| t(11;22)(q24;q11.2) EWS:FLI | Ewing Sarcoma |
| t(21;21)(q22;q22) TMPRSS2:ERG | Prostate Cancer |
| t(4;4)(p16;p16) FGFR:TACC | Glioblastomas |

#### Somatic Mutation

**Somatic mutations per case**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Mutations per Case</th>
</tr>
</thead>
<tbody>
<tr>
<td>medulloblastoma</td>
<td></td>
</tr>
<tr>
<td>leukemia</td>
<td></td>
</tr>
<tr>
<td>breast</td>
<td></td>
</tr>
<tr>
<td>lung</td>
<td></td>
</tr>
<tr>
<td>melanoma</td>
<td></td>
</tr>
</tbody>
</table>

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1.3. Conventional Cancer Therapy

Alkylating Agents - Base damage
- Mustard gas derivatives: Mechlorethamine, Cyclophosphamide, Chlorambucil, Melphalan, and Ifosfamide.
- Ethylenimines: Thiotepa and Hexamethylmelamine.
- Hydrazines and Triazines: Altretamine, Procarbazine, Dacarbazine and Temozolomide.
- Nitrosureas: Carmustine, Lomustine and Streptozocin. Nitrosureas (cross the blood-brain barrier)
- Metal salts: Carboplatin, Cisplatin, and Oxaliplatin.

Topoisomerase Inhibitors and crosslinking agents
- Topoisomerase I inhibitors: Ironotecan, topotecan, other camptothecan analogs
- Topoisomerase II inhibitors: Amsacrine, etoposide, etoposide phosphate, teniposide
- Anthracyclines: Doxorubicin, Daunorubicin, Epirubicin, Mitoxantrone, and Idarubicin.
- Chromomycins: Dactinomycin and Plicamycin.
- Miscellaneous: Mitomycin and Bleomycin.

Anti-metabolites – Nucleotide homeostasis
- Folic acid antagonist: Methotrexate.
- Pyrimidine antagonist: 5-Fluorouracil, Foxuridine, Cytarabine, Capecitabine, and Gemcitabine.
- Purine antagonist: 6-Mercaptopurine and 6-Thioguanine.
- Adenosine deaminase inhibitor: Cladribine, Fludarabine, Nelaarbine and Pentostatin.
- Ribonucleotide reductase inhibitor: Hydroxyurea.
- Enzymes: Asparaginase and Pegaspargase.

“DNA Replication”

Single stand/base lesion ============> Double stand breaks (do NOT activate checkpoints) (active checkpoints)

Micro tubulin/mitotic blocker
- Vinca alkaloids: Vincristine, Vinblastine and Vinorelbine.
- Taxanes: Paclitaxel and Docetaxel.
- Antimicrotubule agent: Estramustine
Overview

1. DNA damage and Cancer
2. Types and Sources of DNA Damages
3. DNA repair pathways
4. DNA damage responses
5. Cell Cycle Checkpoints
6. Special cases
   1. Development DNA breaks - Lymphocyte, Meiosis, neuronal function etc.
   2. Oncogene – how do they induce instability
   3. Telomere, mitochondria and rDNA clusters
   4. Target DNA repair for cancer therapy
2. Types of DNA Damages

Types of DNA Damages:
- Base modifications
  - Base modifications include:
    - Base Akylation
    - Base hydrolysis
    - Abasic site
    - Base Oxidation
- Single Strand breaks (nicks)
- Double Strand breaks
- Replication intermediates
  - Mismatch Repair (MMS)
  - Base Excision Repair (BER)
  - Nucleotide Excision Repair (NER)
  - The Last PART of BER/NER
- Non-homologous end-joining (NHEJ)
- Homologous Recombination (HR)

Factors causing DNA damage:
- Replication Errors
- Oxygen radicals
  - Hydrolysis
  - Alkylating agents
- UV light, chemical agents
- Topo I inhibitors etc
- Development
  - Topo II inhibitors
  - Ionizing Radiation
  - X-ray
- Ionizing Radiation
- X-ray

Mechanisms of DNA repair:
- Mismatch Repair (MMS)
- Base Excision Repair (BER)
- Nucleotide Excision Repair (NER)
- Non-homologous end-joining (NHEJ)
- Homologous Recombination (HR)
2.1 DNA Damage is very common

Table 1. DNA Lesions Generated by Endogenous and Exogenous DNA Damage

<table>
<thead>
<tr>
<th>Endogenous DNA Damage</th>
<th>DNA Lesions Generated</th>
<th>Number Lesions/Cell/Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depurination</td>
<td>AP site</td>
<td>10,000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cytosine deamination</td>
<td>Base transition</td>
<td>100–500&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAM-induced methylation</td>
<td>3meA</td>
<td>600&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7meG</td>
<td>4000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>O&lt;sup&gt;6&lt;/sup&gt;meG</td>
<td>10–30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oxidation</td>
<td>8oxoG</td>
<td>400–1500&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exogenous DNA Damage</th>
<th>Dose Exposure (mSv)</th>
<th>DNA Lesions Generated</th>
<th>Estimated Number Lesions/Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak hr sunlight</td>
<td>—</td>
<td>Pyrimidine dimers, (6–4) photoproducts</td>
<td>100,000/day&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cigarette smoke</td>
<td>—</td>
<td>aromatic DNA adducts</td>
<td>45–1029&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chest X-rays</td>
<td>0.02&lt;sup&gt;f,g,h&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.0008&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dental X-rays</td>
<td>0.005&lt;sup&gt;f,g,h&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.0002&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mammography</td>
<td>0.4&lt;sup&gt;f,g,h&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.016&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body CT</td>
<td>7&lt;sup&gt;f&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.28&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Head CT</td>
<td>2&lt;sup&gt;f,g&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.08&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Coronary angioplasty</td>
<td>22&lt;sup&gt;h&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.88&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tumor PET scan (F&lt;sup&gt;18&lt;/sup&gt;)</td>
<td>10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.4&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;sup&gt;131&lt;/sup&gt;I treatment</td>
<td>70–150&lt;sup&gt;i&lt;/sup&gt;</td>
<td>DSBs</td>
<td>2.8–6&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>External beam therapy</td>
<td>1800–2000&lt;sup&gt;i&lt;/sup&gt;</td>
<td>DSBs</td>
<td>72–80</td>
</tr>
<tr>
<td>Airline travel</td>
<td>0.005/hr&lt;sup&gt;f&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.0002/hr&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Space mission (60 days)</td>
<td>50&lt;sup&gt;i&lt;/sup&gt;</td>
<td>DSBs</td>
<td>2&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chernobyl accident</td>
<td>300&lt;sup&gt;i&lt;/sup&gt;</td>
<td>DSBs</td>
<td>12&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hiroshima and Nagasaki atomic bombs</td>
<td>5–4000&lt;sup&gt;k&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.2–160&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
2.2 Replication Errors

- Up to 100,000 DNA replication origins are available per human cells. Among them, ~30-50,000 are activated in each cell.

- DNA replication requires a clean DNA template, sufficient nucleotide stock, “healthy” polymerase status and proper processing of difficult regions (telomere, centromere, rDNA..etc).

- Oncogene expression could activate DNA replication prematurely, and increases conflicts between transcription and replication.
## 2.3 Polymerase by Numbers

<table>
<thead>
<tr>
<th></th>
<th>Mut/bp (replication)</th>
<th>Genome size (bp)</th>
<th>Mut/genome (replication)</th>
<th>Mut/Generation (Germline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>$10^{-10}$</td>
<td>$3.3 \times 10^9$</td>
<td>$\sim 0.2-1$</td>
<td>$1-4 \times 10^{-8}$</td>
</tr>
<tr>
<td>Mouse</td>
<td>$10^{-10}$</td>
<td>$2.8 \times 10^9$</td>
<td>$\sim 0.5$</td>
<td>$1 \times 10^{-8}$</td>
</tr>
<tr>
<td>Yeast</td>
<td>$10^{-9} \sim 10^{-10}$</td>
<td>$1.3 \times 10^7$</td>
<td>$3 \times 10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>$10^{-9} \sim 10^{-10}$</td>
<td>$5.0 \times 10^6$</td>
<td>$5 \times 10^{-3} \sim 4$</td>
<td></td>
</tr>
<tr>
<td>Virus*</td>
<td>$10^{-3/4} \sim 10^{-5/6} \sim 10^{-7/8}$</td>
<td></td>
<td>$10^{0/1} \sim 10^{-1/-2} \sim 10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>Mito</td>
<td>$\sim 10^{-7}$</td>
<td>$1.7 \times 10^4$</td>
<td>$0.5$</td>
<td>$3 \times 10^{-5}/20$ yr</td>
</tr>
</tbody>
</table>

* RNA virus has the highest mutation rate, followed by retrovirus and DNA virus (about 10 fold drop each step).

Genomic replication polymerase error rate $10^{-8}$ and the repair pathways fix 99% of the breaks.
Mito Polymerase (polG/γ) has a base substitution rate $\sim 2 \times 10^{-6}$

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Taq</td>
<td>$2.3 \times 10^{-5}$/bp</td>
</tr>
<tr>
<td>Pfu</td>
<td>$2.8 \times 10^{-6}$/bp</td>
</tr>
<tr>
<td>Phusion</td>
<td>$4.4-9.5 \times 10^{-7}$/bp (lower with GC buffer, higher with HF buffer)</td>
</tr>
</tbody>
</table>

Fun FACTS: How many E Coli in per ml in overnight mini-prep? $\sim 10^9$
2.4. Developmental DNA breaks

**Lymphocytes**

**Meiosis**

**Neuron synapsis**

**SPO11** is a Topoisomerase II related protein that initiated meiotic recombination by linking itself to DNA in prophase I. Mre11/NBS/RAD50/CtIP complex then cleaves the surrounding DNA to creates DNA double strand breaks, which are repaired by homologous recombination and meiotic specific proteins to result in crossover at the average of 1-2/chromosome.


3. DNA repair pathways

A Brief History of DNA Repair

1930s
- 1947 Recombination in Bacteria
  Lederberg
- 1950s
  - 1953 Double helix structure of DNA
    Crick and Watson
- 1960s
  - 1960 Excision Repair
    in E.coli
  - Mid 1940s
    Photo-dependent DNA repair
    Kelner&Dulbecco
- 1970s
  - 1983 Double holiday junction is proposed by Jack Szostak
  - 1989 Gene targeting
    Carpaccio Evans Smithies
- 1980s
  - Non-homologous end joining is discovered
  - 1967 Cancer predisposing xeroderma pigmentosum was linked to nucleotide excision repair
- 1990s
  - 1990s Synthesis-dependent strand annealing (SDSA)
  - Single strand annealing (SSA)
  - Break induced replication (BIR)
- 2000s
  - Late 90s early 2000s
    Alternative end-joining
  - Regulation between NHEJ and HR
- 2010s
  - 1995 ATM is cloned
  - DNA damage response is recognized
DNA repair pathways

**Base modifications**
- **Excision Repair Pathways**
  - Base Excision Repair (BER)
  - Nucleotide Excision Repair (NER)
  - Mismatch Repair (MMR)
- **Template dependent DNA Synthesis and Gap Filling (Lig1 & Lig3)**

**Single Strand Breaks**
- Direct Fix

**Double Strand Breaks**
- Replication/Transcription
- **Non-Homologues End Joining (NHEJ)**
- Homologous Recombination (HR)
- **Alternative End Joining (A-EJ)/ Micro-homology Mediated End Joining (MMEJ)**
Mismatch Repair (MMR)

MMR is a highly conserved process from prokaryotes to eukaryotes. MMR is often coupled with DNA replication and loading with PCNA ring.

Sensing: travel with DNA polymerase
Strand identification: hemi-methylation in E.coli, potentially nicks in other bugs or eukaryotes.

Function: prokaryote gene: Eukaryotes:
Sensor: MutS = Msh2/Msh6 (MutSα): base substitution/small loops
      Msh2/Msh3 (MutSβ): small/large loop

Helicase/regulator/endo: MutL = Mlh1/Pms1 (MutLα), MutLβ, MutLγ

Scissor: MutH (no eukaryote homology, MutLα is an endonuclease)

Mutations in the human homologues of the Mut proteins affect genomic stability, which can result in microsatellite instability (MI). MI is implicated in most human cancers. Specifically the overwhelming majority of hereditary nonpolyposis colorectal cancers (HNPCC) are attributed to mutations in the genes encoding the MutS and MutL homologues MSH2 and MLH1 respectively, which allows them to be classified as tumour suppressor genes. A subtype of HNPCC is known as Muir-Torre Syndrome (MTS) which is associated with skin tumors.
Nucleotide Excision Repair (NER)

NER is also a highly conserved process from prokaryotes to eukaryotes.

NER is primarily responsible to repair Thymidine Dimer formed following UV lesions.

In bacteria, it is initiated by the scanning the DNA by UvrA-UurB, followed by UvrB loading and UvrC mediated nicking.

In human, there are two kinds of NER pathway that differ at the recognition mechanism term as Globe General NER and Transcription Coupled NER.

Homozygous germline mutations of NER proteins lead to Xeroderma Pigmentaosum (XPA~G), trichothiodystrophy (XPB,XPD, TTDA) and Cockayne Syndrome (CSA and CSB).

XP patients are extremely sensitive to sunlight and develop early on-set basal cell carcinomas. Metastatic malignant melanoma and squamous cell carcinoma are the two most common causes of death in XP patients.
Base Excision Repair (BER)

<-Uracil DNA glycosylase flips a uracil residue out of the duplex, shown in yellow.

While glycosylase and APE homologous are widely spread, the BER pathway is not fully conserved in prokaryotes. Most short patch repair factors were not even found in yeast. BER functions throughout the cell cycle to repair small, non-helix-distorting base lesions (bulky -> NER).

Sensing: Glycosylases
? Long (2-10 nt) vs short (1-2 nt) patch

Members
Sensor: Glycosylase - UNG, OGG1, MAG1, MYH…
Scissor: APE1 (some glycosylase has nickase function)
Polymerases: Polβ, Polλ, Polε, Polδ

Deletion of BER genes increases the mutation rate in a variety of organisms. Somatic mutations in Pol β have been found in 30% of human cancers, and some of these mutations lead to transformation when expressed in mouse cells. Mutations in the DNA glycosylase MYH are also known to increase susceptibility to colon cancer.
# Features of BER/NER/MMR Defects

<table>
<thead>
<tr>
<th></th>
<th>BER</th>
<th>NER</th>
<th>MMR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypersensitive</strong></td>
<td>H₂O₂, Alkylation agents,</td>
<td>UV, Cross linking agents</td>
<td>Nitro, methylating agents</td>
</tr>
<tr>
<td><strong>Accumulate</strong></td>
<td>8-oxo-G, Uracil…</td>
<td>Pyrimidine dimmers</td>
<td>Microsatellite instability</td>
</tr>
<tr>
<td><strong>Cancer</strong></td>
<td>Colon</td>
<td>Skin</td>
<td>Colon/endometria/gastric/ovarian</td>
</tr>
<tr>
<td><strong>Neuronal</strong></td>
<td>Ataxia, microcephaly</td>
<td>Not common</td>
<td>Not common</td>
</tr>
<tr>
<td><strong>Immunology</strong></td>
<td>Antibody defects</td>
<td>mild</td>
<td>Antibody defects</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td>infertile</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DNA repair pathways

**Base modifications**
- Direct Fix

**Single Strand Breaks**
- Replication/Transcription
- Excision Repair Pathways

**Double Strand Breaks**
- Template dependent DNA Synthesis and Gap Filling (Lig1 & Lig3)
- Replication/Transcription

**Excision Repair Pathways**
- Base Excision Repair (BER)
- Nucleotide Excision Repair (NER)
- Mismatch Repair (MMR)

**Non-Homologues End Joining (NHEJ)**
**Homologous Recombination (HR)**
**Alternative End Joining (A-EJ)/ Microhomology Mediated End Joining (MMEJ)**
Homologous Recombination (HR)

HR is conserved in eukaryotes.

Deficiencies in a subset of homologous recombination have been strongly linked to cancer.

Bloom's syndrome, Werner's syndrome and Rothmund-Thomson syndrome are caused by malfunctioning copies of *RecQ helicase* genes involved in the regulation of homologous recombination: *BLM*, *WRN* and *RECQ4*, respectively. In the cells of Bloom's syndrome patients (loss of BLM protein), there is an elevated rate of homologous recombination. Experiments in mice deficient in BLM suggested that the mutation gives rise to cancer through a loss of heterozygosity caused by increased homologous recombination.

Decreased rates of homologous recombination cause inefficient DNA repair, which can also lead to cancer. This is the case with *BRCA1* and *BRCA2*, two tumor suppressor genes whose malfunctioning has been linked with increased risk for breast and ovarian cancer. Cells missing BRCA1 and BRCA2 have a decreased rate of homologous recombination and increased sensitivity to ionizing radiation, suggesting that decreased homologous recombination leads to increased susceptibility to cancer.
Non-homologues end joining

NHEJ is partially conserved in eukaryotes and evolved extensively in vertebrates.

Expressed in all cell types and throughout cell cycles.

Members:
- Ligation: Ku70/86, Lig4/XRCC4/XLF, PAXX
- End-processing: DNA-PKcs, Artemis

Germ line mutations in NHEJ factors lead to microcephaly and severe combined immunodeficiency owing to the requirement of this pathway in V(D)J recombination.

On p53 deficient background, NHEJ deficient mice develop aggressive B cell lymphomas with clonal translocations involving IgH and c-Myc oncogene.

Mutations in the NHEJ pathway is rare in human cancers.
A-EJ and MMEJ are two overlapping pathways that have been implicated in normal DNA repair and in chromosomal translocations.

A-EJ = end joining in cells lacking essential components of the NHEJ pathway (e.g., XRCC4 or KU). MMEJ = end joining events that yield junctions with MH.

The degree of MH at the junctions varies dramatically depending on the sequence context and on the nature of the missing NHEJ factor, suggesting that there might be more than one A-EJ (and likely MMEJ) pathways.

Factors (mostly unknown): CtIP, MRE11, Lig1, PARP and ……

The canonical Ku-dependent NHEJ pathway CAN join DSBs with short MH (usually <4 nucleotides)!!
**Assays for NHEJ/A-EJ/HR Defects**

<table>
<thead>
<tr>
<th></th>
<th>NHEJ</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Junction</strong></td>
<td>Direct or 1-4nt homology</td>
<td>seamless</td>
</tr>
<tr>
<td><strong>Hypersensitive</strong></td>
<td>IR,</td>
<td>IR, CPT, UV, PARPi, crosslink agents</td>
</tr>
<tr>
<td><strong>Accumulate</strong></td>
<td>Chromosome translocations, chromosome break</td>
<td>Replication defects, chromatid breaks</td>
</tr>
<tr>
<td><strong>Cancer</strong></td>
<td>Lymphomas,</td>
<td>Br, Colon, Pancreatic, Ovarian</td>
</tr>
<tr>
<td><strong>Neuronal</strong></td>
<td>Neuronal apoptosis</td>
<td>Not common</td>
</tr>
<tr>
<td><strong>Immunology</strong></td>
<td>SCID</td>
<td>Not common</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td>Infertile, often required for embryonic development</td>
<td></td>
</tr>
</tbody>
</table>
What to do? Pathway choice?
What to do? Pathway choice?

- Resection
  - Yes
  - "end processing"
  - Annealing of MH seqs
  - MMEJ (A-EJ)
    - 1 double SB
      => 2 Single SBs

- NHEJ
Cross Talks before HR and NHEJ

- They are not isolated events AND the pathway choice is not a permanent commitment.
- Share the substrates: DSBs that are not repaired by NHEJ in G1, can leak to S phase and get repaired by HR.
- **CDK1/2 mediated phosphorylation of CtIP plays an important role of regulating end-resection – the first step of HR.**
- Compete for ends: HR starts with end resection and resection (>4nt) will prevents Ku binding and NHEJ. Ku binding to the ends prevent resection by CtIP.
- Regulating each other: BRCA1 actively removes 53BP1 to promote HR. DNA-PKcs and Ku suppresses HR.
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   1. Development DNA breaks - Lymphocyte, Meiosis, neuronal function etc.
   2. Oncogene – how do they induce instability
   3. Telomere, mitochondria and rDNA clusters
   4. Target DNA repair for cancer therapy
4.1. DNA “Damage” Responses

Base modifications

Excision Repair Pathways

Single Strand Nicks

Replication/Transcription

Double Strand Breaks

Base modification and single strand nicks do NOT directly activate DNA damage responses.

Nature Reviews Molecular Cell Biology 14, 197-210
PI3K related kinases (PI3KK)

- Inactivated at the basal level and activated by DNA double strand breaks through their specific “sensing proteins”
  - ATM – MRE11/NBS/RAD50 + dsDNA Breaks
  - ART- RPA/ATRIP+ ssDNA
  - DNA-PKcs – KU70/80 + dsDNA Breaks
- Activated ATM/ATR/DNA-PKcs phosphorylate targeted proteins (>800) at conserved SQ or TQ motifs to modulate checkpoints and DNA repair.
- Mutations in
  - ATM - Ataxia-Telangiectasia Syndrome
  - ATR-Seckel Syndrome
  - DNA-PKcs - SCID with neurological defects.
- Only ATM is inactivated in human cancers at significant levels.
- ATR is essential for normal DNA replication and cellular viability.

ATR: an essential regulator of genome integrity. Nature Reviews Molecular Cell Biology 9, 616-627
DNA Damage Response Pathways

Damage

Sensor

PARP

PI3KKs

Mediators!

Effector

Repair

Check points

Apoptosis

Repair

Cell-cycle arrest

Chromatin remodelling

Recognizing DNA Damages

- ssDNA ends (nicks) are recognized by PARP1
- Extensive single strand DNA – RPA, SSB
- Single stand double stand DNA junction – 9-1-1 complex
- dsDNA ends (15nt) are recognized by Ku70/80, PARP1
- Extensive dsDNA (>100bp) with an end is recognized by MRN(X)
- Other – structural specific nucleases
- Coupling with transcription (NER) or DNA replication (MMR)
- Base alternations – cause strand distortion during globe NER
Damage Responses

- MDC1
- 53BP1
- H2AX
- 53BP1
- ATM
- NBS
- MRE11
- Rad50
- H2AX

DNA-PKcs

ATM

ATR

PI3K
ATM mutations lead to cancer

- Mutations of ATM and its downstream checkpoint components increased the risk for cancer
- Mutation of the repair specific substrates of ATM are not common in cancers.
Overview

1. DNA damage and Cancer
2. Types and Sources of DNA Damages
3. DNA repair pathways
4. DNA damage responses
5. Cell Cycle Checkpoints
6. Special cases
   1. Development DNA breaks - Lymphocyte
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Normal Cell Cycle Progression

- Cyclin B/Cdk1
- Cyclin A/Cdk2
- Cyclin D1/Cdk4/6
- Cyclin E/Cdk2

Growth Factors
- Wee1
- Cdc25
- ↑ Cyclin A/Cdk1 (Cdc2)
- ↑ Cyclin B/Cdk1 (Cdc2)
- ↑ Cyclin D1/Cdk4/6
- ↑ Cyclin E/Cdk2

Phases:
- G1
- S
- G2
- M

Regulatory Factors:
- Rb
- E2F
- CCRE

Graph showing concentration changes over phases: Cyclin D, Cyclin E, Cyclin A, and Cyclin B.
Cell cycle and Checkpoints

**G2/M checkpoint:** licensing for mitotic entry. Exp: accumulation of G2 fraction, reduce mitotic cells.

**Spindle checkpoint:** permit division – all chromosomes are aligned correctly.

**G1/S checkpoint:** Just before entry into S phase, making the key decision of whether the cell should divide, delay division, or enter a resting stage. Exp: reduce S phase content, reduce DNA synthesis.

### Cyclin A/B/Cdk1 (Cdc2)

Cdc25 → Cyclin A/B/Cdk1 (Cdc2)

↑ Chk1 → Chk2

↑ ↑

ATM/ATR

### Cyclin D1/Cdk4/6

Cyclin D1/Cdk4/6 → Ink4a/p16

↓ Cip/p21

↓ p53

↓ Chk2

↑ ATM/ATR

### Cyclin E/Cdk2

### Intra-S phase “checkpoint”:
Availability of nucleotides, progression of DNA replication

Ser10p-H3

PI/DNA

Ctrl 1 hr after IR

BrdU

PI/DNA

Ctrl 12 hr after IR 5 Gy

Rb

E2F

PI/DNA

Ctrl

1 hr after IR

G1

G2

M

S

I

3o

Exp: reduce S phase content, reduce DNA synthesis

G1/S checkpoint: Just before entry into S phase, making the key decision of whether the cell should divide, delay division, or enter a resting stage. Exp: reduce S phase content, reduce DNA synthesis.
4.1. DNA “Damage” Responses

Base modifications

- Excision Repair Pathways

Single Strand Nicks

- Replication/Transcription

Double Strand Breaks

Single strand breaks do NOT activate DNA damage response. But extended single strand DNA will! (through ATR)

Repair

- chromatin
- transcription

....

Cell cycle Checkpoints

Cell Death
Aneuploidy – many copies
Leaky spindle checkpoint lead to 1) anaphase bridge; 2) increased aneuploidy.

- Activation of Spindle Checkpoint is achieved by Cdc20 that usually prevents cyclin B degradation and keeps securin inactive.
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6.1 Lymphocytes Development

**Pro B**
- $D \cdot J_H$
- $V-DJ_H$

**Pre B**
- $V \cdot J_{\kappa/\lambda}$

**Immature B**

**Mature B cell**
- IgM

**Antigen Stimulation** (Helper T-cell)
- Class Switch Recombination
- Somatic Hyper Mutation
- IgG, IgA, IgE

**Bone Marrow**

**Germinal Center**

**V(D)J Recombination**

**Class Switch Recombination and Somatic Hypermutation**

**RAG1/2**

**AID**
6.1.2 Lymphocyte to Lymphoma

**V(D)J Recombination**
- Non-homologous end joining
- DNA damage response (ATM)
- Mistakes -> Translocations
- IgH (IgL) - cMyc (Burkett’s Lymphomas)
- IgH- Bcl2 – Follicular Lymphomas
- IgH- CyclinD1 – Mantle Cell Lymphomas

**Class Switch Recombination**
- Non-homologous end joining
- Alternative – end joining
- DNA damage response (ATM)
- Mistakes -> Translocation
- IgH – Bcl6 (DLBCL)

**Somatic Hypermutation**
- Mismatch Repair/BER
- Base excision Repair
- Mistakes-> Mutation of other genes
- Myc
- Bcl-6
- …..
6.1.3 Translocation – risk factors

- **Breaks!**
- **Reduced repair fidelity**
- **Rapid proliferation and/or accumulation of several “oncogenic” events**
- **Defective “checkpoints”**

[Diagram showing the progression from normal to cancer, including oncogene upregulation, tumor suppressor downregulation, translocations, loss of checkpoints, and the resulting residual, relapse, disseminate, metastasis states.]
6.1.4 Translocation –where to go?

- Random translocation followed by functional selection
  - Passenger mutations/genomic instabilities
  - Why c-myc, not N-myc or L-myc?
- Targeted: Cryptic recombination site
- Other “influencing factors”: transcription, physical distance, nuclear structure, etc.
  - Break first vs proximity first!


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   3. Telomere, mitochondria and rDNA clusters
   4. Target DNA repair for cancer therapy
6.2. Oncogene induced genomic instability

1. Premature DNA replication - not enough transcription to clear out the inappropriate replication origin

2. Overall increased transcription (e.g. Myc)- more conflicts between transcription and replication.

3. Replication with limited recourse: reduced/unbalanced nucleotide pools. Especially excess ribonucleotides

4. Energy deprivation – lack of Glutamine and Glucose
Overview

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6.2.1 Telomere is the end of chromosome

Linearized eukaryotic chromosome present special “end” problems.

• A specialized mechanism of duplication – single replication origin

• To be protected from the cellular machinery that detects and repairs DNA breaks.
Telomere is protected from repair mechanisms by Shelterin complex

How telomeres solve the end-protection problem.

de Lange T.
Shelterin Protect Telomeres
6.2.2 Mitochondria and ROS

- **Reactive oxygen species** (ROS) are chemically-reactive molecules containing oxygen. Examples include oxygen ions and peroxides.
- ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling.
- ROS could directly modify DNA, RNA and proteins.
- It is also a mature source for mitochondrion DNA damage.
- ROS are also generated by exogenous sources such as ionizing radiation.

6.3.2 Mitochondrial DNA Damage

- Mitochondrial DNA (mtDNA) exists in multiple copies, and is tightly associated with a number of proteins to form a complex known as the nucleoid.

- Inside mitochondria, reactive oxygen species (ROS), or free radicals, byproducts of the constant production of adenosine triphosphate (ATP) via oxidative phosphorylation, create a highly oxidative environment that is known to damage mtDNA.

- A critical enzyme in counteracting the toxicity of these species is superoxide dismutase, which is present in both the mitochondria and cytoplasm of eukaryotic cells.

- Recent studies also identified the mitochondrion form of Lig3 as a critical component for mitochondrion DNA repair and survival.


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Genotoxic Drugs used in Cancer Chemotherapy

- **Alkylating agents**: The first class of chemotherapy agents used. These drugs modify the bases of DNA, interfering with DNA replication and transcription and leading to mutations.
- **Intercalating agents**: These drugs wedge themselves into the spaces between the nucleotides in the DNA double helix. They interfere with transcription, replication and induce mutations.
- **Enzyme inhibitors**: These drugs inhibit key enzymes, such as topoisomerase, involved in DNA replication inducing DNA damage.
BRCA2 deficient cells are hypersensitive to PARP inhibitors

- There are 17 members in the PARP super family.
- They share a conserved domain that presumably mediated NAD and ATP-dependent poly ADP ribosylation activity.
- Only PARP1 and PARP2 activity were shown to be activated by DNA strand breaks – both single and double.
- The activation of PARP1/II contribute to DNA repair by at least three known mechanisms:
  - PARP1/II directly interacts with XRCCI-Lig3 complex to recruit them to DNA
  - Add PAR to H2A and H2B to physically open up chromatin
  - The PAR chains created at site of DSB could serve as an anchor to recruit DNA repair proteins, including NBS, BARD and others.

Reviewed in a special issue of Mol Cell in June 18, 2015
6.4.2 How does PARP “inh” work?

- Prevent PARylation mediated BER increase single stand nicks that are converted to double stand breaks during replication.
- PARP inhibitor traps PARP1 at the site of DNA damage, where it blocks DNA replication and transcription. **Inhibition ≠ loss of enzyme in DNA repair**

<table>
<thead>
<tr>
<th></th>
<th>ATM</th>
<th>DNA-PKcs</th>
<th>ATR</th>
<th>PARP1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>Viable</td>
<td>Viable</td>
<td>Lethal</td>
<td>Viable</td>
</tr>
<tr>
<td>Catalytic Inactivation</td>
<td>Lethal</td>
<td>Lethal</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Inhibitors</td>
<td>Withdraw from Phase I due to toxicity</td>
<td>- (not specific)</td>
<td>Well tolerated</td>
<td>FDA approved</td>
</tr>
</tbody>
</table>

- PARP inhibitors block the recycle of NAD+ during replication and DNA damage responses.
- PARPi affects chromatin organization to impair DNA repair
DNA damage and repair are constant battles in all living cells.

DNA repair play important roles in the initiation, treatments and therapeutic responses of cancer.

DNA damage response activates the cell cycle checkpoints.

Cell cycle checkpoints promote accurate repair of DNA damages.

Checkpoints also act as the gate keeper to prevent damaged cells from further proliferation.

“The DNA damage response in tumorigenesis and cancer treatment” & “Genomic instability in cancer” Nature Review Cancer
Additional references
### DNA repair and DNA damage response defects

#### Problems In Responding to DNA Damage Or Stalled Replication Forks

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia telangiectasia</td>
<td><strong>ATM</strong> detects DNA damage &amp; stalled forks</td>
</tr>
<tr>
<td>Seckel syndrome</td>
<td><strong>ATR</strong> detects DNA damage &amp; stalled forks; other checkpoint/replication genes may also be involved</td>
</tr>
<tr>
<td>Li-Fraumeni syndrome</td>
<td><strong>CHK2</strong> and <strong>TP53</strong> respond to DNA damage and stalled forks</td>
</tr>
<tr>
<td>Ataxia telangiectasia-like disorder</td>
<td><strong>MRE11</strong> rescues stalled forks; repairs DNA damage</td>
</tr>
<tr>
<td>Nijmegen breakage syndrome</td>
<td><strong>NBS1</strong> rescues stalled forks; repairs DNA damage</td>
</tr>
<tr>
<td>Bloom syndrome</td>
<td><strong>RECO2/BLM</strong> encodes a DNA helicases that rescues stalled forks</td>
</tr>
<tr>
<td>Werner syndrome</td>
<td><strong>RECO3/WRN</strong> encodes a DNA helicase</td>
</tr>
<tr>
<td>Rothmund-Thomson syndrome</td>
<td><strong>RECO4/RTS</strong> encodes a DNA helicase</td>
</tr>
<tr>
<td>Rapadilino Syndrome</td>
<td><strong>RECO4</strong> encodes a DNA helicase</td>
</tr>
</tbody>
</table>

#### Problems In Repair Of Damaged DNA

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial breast cancer, Ovarian,</td>
<td><strong>BRCA1</strong> and <strong>BRCA2</strong> repair radiation-induced breaks in double-stranded DNA</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>Eleven FA genes, one of which (D1) is <strong>BRCA2</strong>, ICL repair</td>
</tr>
<tr>
<td>Xeroderma pigmentosum</td>
<td><strong>XPA</strong>, <strong>XPC</strong>, <strong>XPF</strong>, <strong>XPG</strong> repair nucleotide excisions</td>
</tr>
<tr>
<td></td>
<td><strong>XPD</strong> is a DNA helicase</td>
</tr>
<tr>
<td>Xeroderma pigmentosum variant</td>
<td><strong>POLH/DNA polymerase-eta</strong> carries out trans-lesion DNA synthesis</td>
</tr>
<tr>
<td>Cockayne syndrome</td>
<td><strong>CSA</strong> and <strong>CSB</strong> repair DNA damage</td>
</tr>
<tr>
<td>XP-Cockayne syndrome</td>
<td><strong>XPD</strong> encodes a DNA helicase</td>
</tr>
<tr>
<td>Trichothiodystrophy</td>
<td><strong>XPB</strong> and <strong>XPD</strong> encode DNA helicases</td>
</tr>
<tr>
<td>HNPCC, hereditary non-polyposis colon cancer</td>
<td><strong>MSH2</strong>, <strong>MLH1</strong> (major), <strong>MSH6</strong>, <strong>PMS2</strong>, <strong>PMS1</strong> (minor) involved in mismatch repair (MMR)</td>
</tr>
<tr>
<td>LIG4 syndrome</td>
<td><strong>LIG4/DNA ligase IV</strong> is required for non-homologous DNA end-joining</td>
</tr>
<tr>
<td>Radiosensitive severe combined immunodeficiency (RS-SCID), Omenn Syndrome</td>
<td><strong>ARTEMIS</strong> encodes a hairpin-specific nuclease that plays a subsidiary role in non-homologous end-joining, and V(D)J recombination.</td>
</tr>
</tbody>
</table>
# Types of DNA Damages

## Strand Breaks
- Single Strand breaks
- Double strand breaks

## Replication
- Transcription
- Repair intermediates

## Base modifications

<table>
<thead>
<tr>
<th></th>
<th>Abasic site</th>
<th>Mismatch</th>
<th>Thymidine Dimer</th>
<th>Cross link</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base hydrolysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base Aklylation</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Base Oxidation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base adduction</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

- Cytosine
- Uracil
- Thymine
- 5-methylcytosine
- Thymine
- Methylguanine
- O-6-methylguanine
- Thymine
- Guanine
- O-4-methylthymine
- Thymine
- Thymine
- Thymine
- Thymine
- Thymine
- Thymine
- Thymine
- Thymine