DEFINITION

An intracellular process responsible for recognizing errors in DNA strands and correcting the error by means of enzymatic and chemical reactions in order to prevent occurrence of permanent mutations and / or cell death
COMMON CAUSE

1. Intrinsic factor = DNA replication error, deamination / depurination, spontaneous mutation

2. Extrinsic factor = UV light, ionizing radiation, mutagen (such as alkylating agent, adduct donor), clastogen (topo II inhibitor), inflammatory stimulus
TYPES OF DNA DAMAGE

- single-strand break
- damaged base
- double-strand break
- mis-match
- intra-strand crosslink
- inter-strand crosslink
REPLICATION ERROR (MISMATCH)

- Replication is a crucial process in interphase phase in preparation of 4n DNA ready for division prior to M phase
- DNA polymerase has immense but not perfect proofreading capacity
- Error rate of 1 in 10,000,000 bases is expected, 99% of these are corrected
- Tandem repeat DNA can pose more difficulty during replication and slippage of replication machinery commonly introduces error in number of DNA repeat
- Permanent mutation occur in daughter strands in the next round of replication in unrepaired DNA
- Consequence of a permanent mutation can be deleterious to cell function commonly leading to cell death or misdivision
CONSEQUENCE OF REPLICATION ERROR

DNA ready for replication

- G C
- T A
- C G
- A T
- G C
- T A

A mistake was made during replication

- G C
- T A
- C T
- A T
- G C
- T A

If not corrected, a permanent change will be passed on when the strand on the right is copied

- G C
- T A
- A T
- A T
- G C
- T A
DNA OXIDATIVE DAMAGES

- Occur in ~ 10000 lesions / cell / day
- 8-oxo-guanosine is most deleterious
- 8-oxo-guanosine is mispaired with adenine
- Normal G:C -> oxoG:A -> T:A
- Uncorrected lesion will cause G to T transversion
- Requires base excision repair
DIRECT CHEMICAL DAMAGE

• Depurination - the base is simply ripped out of the DNA molecule leaving a gap
• Deamination - An amino group of Cytosine is removed and the base becomes Uracil
• Deamination - An amino group of Adenine is removed and the base becomes Hypoxanthine
• Thymine dimer formation after exposure to UV-B
Random photons of ultraviolet (UV) light induce aberrant bonding between neighbouring pyrimidines (thymine & cytosine) bases on the same strand of DNA. The will prevent the replication machine from duplicating the DNA. The cell will die!
COMPONENTS OF REPAIR SYSTEM

1. Sensor: recognition of error
2. Transducer: induction of repair protein / machinery
3. Effector: execution of actual repair

The cellular DNA damage response

- DNA damage
- Sensors
- Transducers
- Mediators
  - Cell cycle arrest
  - DNA repair
  - Apoptosis
- Effectors
SENSOR MECHANISM

DSB
Damage response protein complex

Sensors/mediators:
- MRE11/RAD50/NBS1, H2AX, MDC1, BRCA1, 53BP1, RNF8, RNF168
- miR-24

Transducers:
- miR-421
  - ATM, ATR

Effectors:
- DNA repair
- Cell cycle checkpoint
- Apoptosis

NHEJ
- Ku70/80
- DNA-PK
- Artemis
- XLF
- XRCC4
- LIG4

HRR
- CtIP
- BRCA1
- RAD52
- RAD51A
- RAD51B
- RAD51C
- RAD51
- BRCA2
- RAD54

G1/S
- CHK1
- CHK2
- p53
- MDM2
- p21
- Cyclin E(A)
- CDK2
- miR-124a

Intra-S
- CHK1
- CHK2
- Cyclin E(A)
- CDK2
- CDC25A

G2/M
- CHK1
- CHK2
- p53
- miR-504
- FAS
- PUMA
- BAX
- NOXA

miR-21
miR-449a/b
miR-16

miR-210
miR-373
miR-124a
miR-373
miR-124a
miR-21

This diagram illustrates the sensor mechanism for DNA double-strand breaks (DSB) repair pathways. The core protein components of various pathways are highlighted, showing how miRNA regulation affects the cellular response to DSB damage.
A. DIRECT REPAIR

- Enzyme, photoactivation repair enzyme (PRE) absorbs a photon of light (from blue light) and is able to cleave the bond forming the thymine dimer.
B. BASE EXCISION REPAIR

- For correction of specific Chemical Damage in DNA
  - Uracil
  - Hypoxanthine
  - 3-m Adenine
  - Urea
  - Formamidopyrimidine
  - 5,6 Hydrated Thymine
BASE EXCISION REPAIR
LONG PATCH BASE EXCISION REPAIR
C. NUCLEOTIDE EXCISION REPAIR AND SSB REPAIR SYSTEM

• Used by the cell for bulky DNA damage such as thymine dimer and crosslinks

• Non specific DNA damage
  • Chemical adducts …
  • UV photoproducts

First identified in 1964 in E.coli.
NUCLEOTIDE EXCISION REPAIR
D. MISMATCH REPAIR SYSTEM

• A highly conserved eukaryotic machinery to repair single base mismatch or mismatch of tandem repeat DNA such as microsatellites

• Several DNA repair and tumor suppressor genes contain such repeat
D. MISMATCH REPAIR SYSTEM IN E.COLI
EUKARYOTIC MMR

• **Step 1** Initiation of MMR begins when a heterodimer of the MutS homolog (MSH), either MSH2–MSH6 (MutS/) or MSH2–MSH3 (MutSb), binds to mismatched DNA.

• **Step 2** Following mismatch or IDL recognition, a heterodimer of MutL homolog (MLH) consisting of MLH1 and the post-meiotic segregation 2 protein (PMS2) is recruited to MutS/or MutSb

• **Step 3** The ‘DNA clamp’ proliferating cell nuclear antigen (PCNA) is loaded by replication factor C (RFC), after which PCNA interacts with MLH1–PMS2 (MutL/) to enable PMS2 to exert its endonuclease activity

• **Step 4** Excision of mismatched DNA is mediated by a well-characterized exonuclease 1 (Exo1)-dependent interaction

• **Step 5** Replication protein A (RPA) binds to and protects the single-stranded DNA that is generated by the exonuclease, and promotes excision termination

• **Step 6** Lastly, the excised bases are filled in by DNA polymerase d and ligated by DNA ligase
E. STRAND BREAK REPAIR SYSTEM

- Homology directed repair
  Homologous recombination (HR)
  RNA-intermediate based repair
- Non-homology directed repair
  Non-homologous end joining (NHEJ)
  RNA-based repair
DSB REPAIR BY HR
DSB REPAIR BY NHEJ
RNA- MEDIATED DSB REPAIR

http://www.devbio.biology.gatech.edu
SSB and DSB Repair Proteins
DNA REPAIR SYSTEM INVOLVED IN CANCER CHEMOTHERAPY INDUCED DAMAGE

- Alkylating agent: direct repair, MMR, BER
- Topo II inhibitor: DSB repair
- Platinum: NER
- Radiation: DSB repair
CONSEQUENCE OF INTRODUCTION OF MUTATION INTO DNA STRAND AND GENES

- Loss of function (haploinsufficient, hypomorphomic and null allele)
- Gain of function
- New function (neomorphomic)
- No change in function (synonymous mutation or SNP)
Sporadic Dysfunction Repair System

Common occurrence in non-hereditary cancers
GERMLINE DYSFUNCTIONAL REPAIR SYSTEM

GENETIC DISORDERS WITH CANCER PREDISPOSITION
NER - XERODERMA PIGMENTOSUM, TRICHOTHIODYSTROPHY, COCKAYNE SYNDROME

• Xeroderma Pigmentosum

Moriz Kaposi (1874) used this term for the first time to describe the symptoms observed in a patient. XP patients exhibit an extreme sensitivity to sunlight and have more than 1000-fold increased risk to develop skin cancer, especially in regions exposed to sunlight such as hands, face, neck.
NER - COCKAYNE SYNDROME

• A second disorder with UV sensitivity was reported by Edward Alfred Cockayne in 1936.

• CS is characterized by additional symptoms such as short stature, severe neurological abnormalities caused by dysmyelination, bird-like faces, tooth decay, and cataracts. CS patients have a mean life expectancy of 12.5 years but in contrast to XP do not show a clear predisposition to skin cancer. CS cells are deficient in transcription-coupled NER but are proficient in global genome NER.
NER - TRICHTHIODYSTROPHY

• TTD, literally: “sulfur-deficient brittle hair”, was reported by Price in 1980.

• In addition to symptoms shared with CS patients, TTD patients show characteristic sulfur-deficient, brittle hair and scaling of skin.

• This genetic disorder is now known to correlate with mutations in genes involved in NER (XPB, XPD, and TTDA genes). All of these genes are part of the 10-subunit transcription/repair factor TFIIH, and TTD is likely to reflect an impairment of transcriptional transactions rather than regular defect in DNA repair. This disorder is therefore sometimes referred as a “transcriptional syndrome”.

MMR – LYNCH SYNDROME

- HNPCC is a cancer predisposition multi-cancer syndrome with the highest prevalence
- Colon, Endometrium, KUB, GI, GBM, sarcoma, sweat / sebaceous CA
- Variants: Turcot syndrome, Muir-Torre syndrome
- Due to 4 common MMR gene mutation and 1 deletion
- hMSH2, hMLH1, hMSH6, hPMS2, EPCAM deletion
MMR DEFICIENT

- Can be demonstrated by MSI test or IHC stain for MMR protein
- MSI-H = possible HNPCC
  - BRAF V600E + = sporadic CA
  - BRAF V600E - = sporadic or HNPCC
  - MLH1 promoter methylation
- MSI-L = sporadic CA
- MSS = sporadic CA
Complete loss

Clonal or Zonal loss
MSI Testing Schematic

MSI Testing

MSI-High

MSI-Low or MSS

CRC <50 OR multiple primaries OR family history of LS-related cancers

CRC >50 and no family history

Refer to Genetics

KEY: BLUE = Testing/Consultation; GREEN = Results/History
IHC Testing Schematic
(With BRAF and Hypermethylation)

IHC Testing

- All proteins present (Negative IHC result)
  - CRC <50 OR multiple primaries OR family history of LS-related cancers
  - CRC >50 and no family history

- Absent MSH2 & MSH6, or MSH6 or PMS2
  - BRAF and Hypermethylation Negative
  - BRAF and Hypermethylation Positive
  - Refer to Genetics

- Absent MLH1 & PMS2
  - BRAF and Hypermethylation Testing

- Indeterminate IHC
  - MSI or IHC on another tumor
    - MSI-High
    - MSI-Low or MSS
    - Suspicious family history

KEY: BLUE = Testing/Consultation; GREEN = Results/History
Multi Society Task Force
Universal Testing of CRC for dMMR

1. MSI testing
2. MSI-high
3. Normal
4. No further testing
5. Presence of BRAF mutation (and/or presence of MLH1 promoter hypermethylation)
6. Absent BRAF mutation (and/or MLH1 promoter hypermethylation)

Colorectal cancer surgical specimen → IHC testing → Loss of MHL1 & PMS2 → BRAF testing (and/or promoter hypermethylation testing)
→ Absent BRAF mutation (and/or MLH1 promoter hypermethylation)
→ Refer to genetic counseling for consideration for germline testing (guided by IHC testing results)
→ Loss of other MMR proteins

Giardiello FM, Am J Gastro 2014;109:1159
Colorectal cancer

### MSI testing
- **MMR proficient (85%):**
  - ~77.5%
  - Stable (MSS)
  - Normal
  - Wild type
- **MMR deficient (sporadic and Lynch) (15%):**
  - ~7.5%
  - Unstable (MSI high)
  - Loss
  - Mutant
  - Unstable (MSI high)
  - Loss
  - Wild type

### IHC for MLH1, MSH2, MSH6, and PMS2
- Stable (MSS)
- Normal
- Wild type

### BRAF mutation testing
- Mutant
- Mutant

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If metastatic, perform KRAS mutation analysis:
- Likely sporadic CRC
- Likely sporadic CRC, poor prognosis
- Sporadic CRC, reduced 5-FU benefit

- If MSH2, MSH6, and/or PMS2 loss, perform sequencing to confirm Lynch
- If MLH1 loss, perform MLH1 promoter methylation. If unmethylated, perform sequencing to confirm Lynch
- Good prognosis, reduced 5-FU benefit
Bloom syndrome

- Head is disproportionately small
- Striking paucity of subcutaneous fat tissue throughout infancy and childhood, and
- A redness of the cheeks and nose that characteristically makes its appearance in infancy after sun exposure.
- Chronic obstructive lung disease, Diabetes mellitus and malignancies of varied types are some of the common complications of Bloom syndrome.
Fanconi Anemia

Defect in DNA repair
Autosomal Recessive

Increased risk for AML, solid tumors & aplastic anemia

Absence or hypoplastic thumb

Macrocytic Anemia
Elevated fetal hemoglobin

- Short stature
- Low set ears, deafness
- Strabismus
- Skin hypopigmentation
- Renal abnormalities
- Rx: Bone marrow transplant
DNA REPAIR PATHWAY AS A CANCER THERAPEUTIC TARGET

• Chemical Induction of repair deficiency to promote chemosensitivity and radiosensitivity “BRCAness”
• Synthetic lethality
• Immunotherapy in MMR tumor
• miRNA modulated DDR pathway: using or targeting miRNA to enhance chemosensitivity and reverse chemoresistance
BRCANESS

- The hallmarks of BRCAness is elevated genomic instability and deficient HR pathway activity
- Hypersensitive to alkylating, platinum-based chemotherapies that generate DNA interstrand crosslinks and induce double-stranded DNA breaks during crosslink removal
- Not sensitive to mitotic spindle poisons such as the taxanes and vincristine. This is because spindle disruption caused by these agents can induce apoptotic cell death in BRCA-proficient but not in BRCA-deficient tumors.

Calculation of sample-specific BRCA score

Cancer type classification
- BRCA-like
- non BRCA-like

Genome prediction
- Instability
- Stability

Prognosis prediction
- BRCA-like
- non BRCA-like

Chemotherapy prediction

Prognosis prediction
- BRCA-high
- BRCA-low
Synthetic lethality exploits inter-gene relationships where the loss of function of either of two related genes is nonlethal, but loss of both causes cell death [BRCA1/2 and PARPI inhibitor]
**Absence of immunotherapy**

- Mismatch repair deficiency
- Frameshift mutations
- Protein with mutation-associated neoantigen (MANA)
- Tumor cell
- PD-L1/PD-1 interaction blocks T-cell activation
- T-cell anergy

**Presence of anti-PD-1**

- Mismatch repair deficiency
- Frameshift mutations
- Protein with mutation-associated neoantigen (MANA)
- Tumor cell
- PD-L1/PD-1 interaction blocked by antibody, freeing T cell to kill tumor cell
- T-cell activation

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LIMITATION OF TRANSLATIONAL RESEARCH ABOUT DNA REPAIR

- Beside PARP1 inhibitor, no other repair based strategy has been clinically approved
- DNA repair cannot be simply and directly measured in clinical in vitro testing currently available
- Most available tests are for viability