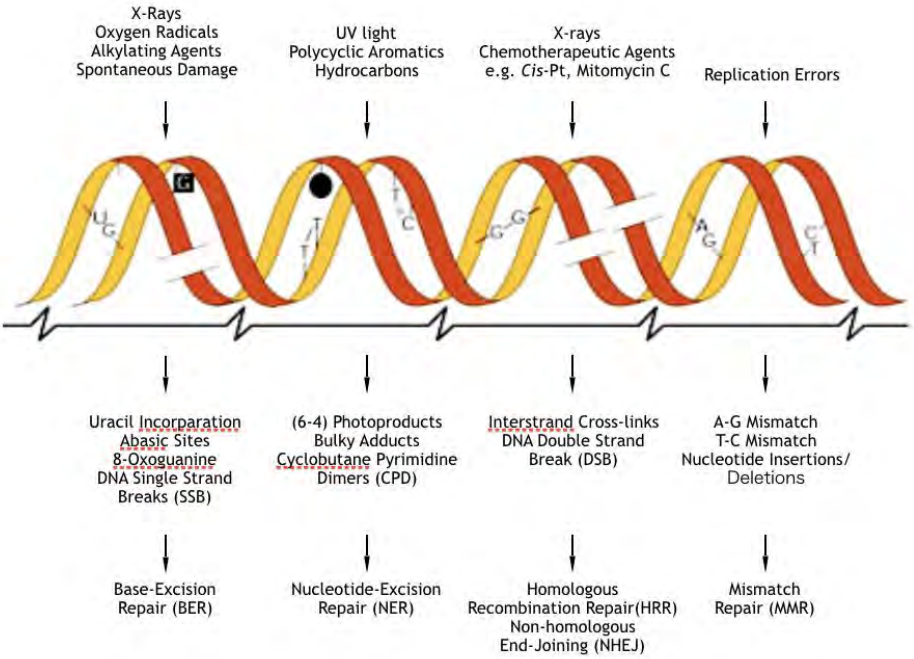
AMEG
Use the Learning Objectives for Exam Preparation

Lecture 1: The DNA Damage Response: DNA Recognition

A cell has about 100,000 DNA lesions induced in its DNA per day without the presence of radio- and chemotherapy.

Types of DNA Damages

1. DNA Double Strand Breaks – Caused by X-rays, Chemotherapy
2. Intra-strand Cross Links – Caused by UV Radiation
3. Ionising Radiation – can induce damage to the bases of the DNA and the process of DNA replication itself
4. Base Mismatches
5. Mis-Modification of individual Bases

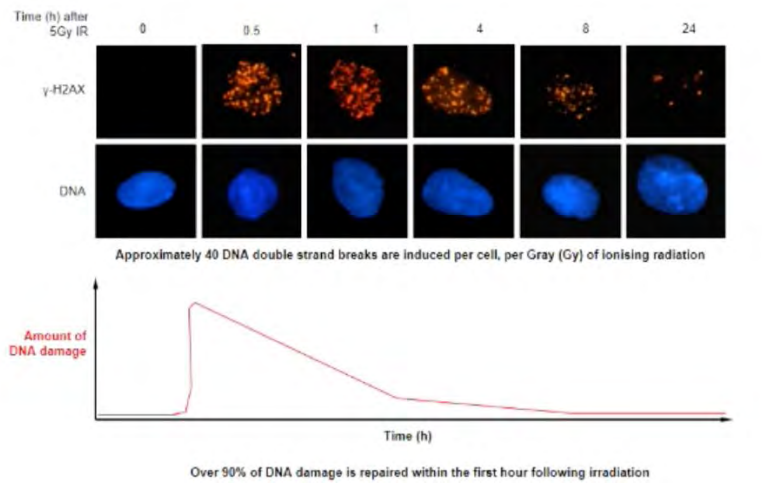


DNA Double Strand Breaks provide the most cytotoxic niche. They are resolved by 2 mechanisms: Non-Homologous End-Joining (NHEJ) and homologous Recombination (HR).

Why is DNA Repair Important?

- Even 1 unrepaired DSB is sufficient enough to kill the cell. The DNA can lose bits of DNA since it cannot be packaged into chromatin
- Defects in the DNA repair pathways strongly contribute to tumorigenesis e.g. chromosomal translocations
- DNA damage can be induced by exposure to agents such as: Ionizing radiation, X-rays, Chemotherapeutic Drugs

We use immunofluorescence and fluorescent markers of DSBs to see how long fluorescent markers take to disappear. We take cells irradiate them, then we fix them with 3.6% paraformaldehyde (PFA). We permeabilise them with TX-100 (detergent). This allows the antibodies to get into the cell. We then add specific antibodies to the DNA repair protein of our choice e.g. anti-RAD51. Then we apply a secondary antibody which is coupled to a fluorescent tag. We take these cells and stain them with DAPI, which recognises DNA. We then look at the results under a fluorescent microscope.



Example: Primary antibodies are specific to γ-H2AX, which is a specific marker of DSB. Each dot represents a single DSB, so per grade of ionising radiation, we induce about 40 DSB per cell. After irradiation, there is a huge number of damage and over time the number of DSB reduces. This indicates that repair processes are efficient. About 90& of DSBs are repaired within the first couple of hours. Once all DSBs are repaired, the cells can recover and turn off all checkpoints and start undergoing the cell cycle again. The resolution and the timing the γ-H2AX foci arrives and goes away indicates how good the cells are at DNA repair processes.

2 Repair Pathways:

1) NHEJ:
- Can occur at any phase of the cell cycle. This is because it does not require a homologous template
- Accurate but small deletions can occur at DSB repair sites. NHEJ is not an error prone process
- It is essential for cell viability, so without it the cell will not be viable
- Required for immune system development and maturation: formation of immunoglobulins and T cell receptor
- Inherited defects in NHEJ are associated with RS-SCIDD (radio-sensitivity) and/or severe growth deficiency.

Proteins involved:

1. DNA-PKcs
2. Ku Heterodimer
3. Artemis
4. DNA Ligase IV
5. XRCC4
6. XLF

2. Fragment and Denature them
3. Anneal them to chips that have different SNPs on them
4. Leave the DNA on 37 degrees and read out the fluorescent signal
5. Patient DNA should anneal but only if it has the corresponding variation (if SNP is not present = faint yellow present. If SNP is present, bright yellow light)

Dual colour readout: We have SNPs and the missing base will be colour coded.
This tells you about the conserved regions between the affected and unaffected regions

Why use an SNP Array?

- Cheap and powerful
- Robust: Much less laborious and less error prone than using other markers
- With online biomarker databases, we not have a good idea of the genetic locations of 1,000,000 SNPs – enormous coverage
- Commercial libraries of SNPs are easily available
- We can tell from the data there is: Inheritance and Mutation Mapping

GWAS: Genome Wide Association Study:

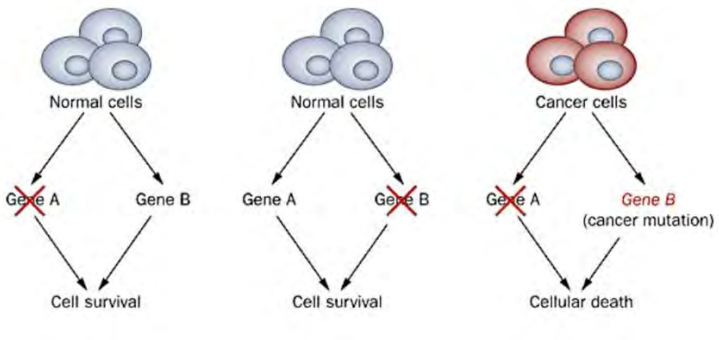
- It can correlate disease or susceptibility (or any other trait) with SNPs
- It is used to discover new gene variants associated with a particular phenotype/ disease

Using comparative genome arrays (aCGH) to analyse whole genome changes:

1. Take genome from control and patient
2. Label them with different colours and mix them together and hybridize them in a slide
3. Look at the differences in the amount of DNA in the two samples. You can pick out the Mutation

If the segment of DNA is present only in the control - the dot is red. If the segment is present only in the patient – dot is green. If there is a mutation – the dot will be yellow

Exome sequencing can also be done. This is sequencing of mRNA. This will only pick up protein changes



Cancer Therapy by Synthetic Lethality:
In replicating cells, HR serves as a backup repair pathway of DDB. Inhibition of SSB repair increases the HR activity. SSB repair is a small DNA repair pathway that requires a few proteins, on of these proteins are called PARP1.
PARP1 enzyme geos to SSB, modified into polynucleotide ribose

PARP1 inhibitors e.g. Olaparib
With these PARP inhibitors, the replication fork collapse in cancer cells due to SSB. The collapsed forks will depend on HR and RAD51/BRCA1 and BRCA2 for repair.

Use of this:
Subsets of familial cancers have mutations in BRCA1/2 and FANCN and FANCJ. In cells with BRCA1/2 mutations, the cells cannot undergo HR and so the cancer cells die of unrepaired spontaneous SSB.

Cancer therapy by Synthetic Lethality: BRCA2
If you treat cells with BRCA2 with PARP inhibitor, then they do not die, but the cells without BRCA2 die.

Cancer therapy by Synthetic Lethality: BRCA1/ BRCA2
Therapy is specific to the tumors this is because the normal tissue of the cancer is heterozygous for the mutations in BRCA1/2. This means that the normal cells are not sensitive to PARP inhibitors because they can use HR as a backup pathway. Cancer cells usually lose BRCA1 or BRCA2 function, so cancer cells are sensitive to PARP inhibitors

Property	ZFNs	TALENs	RGENs
Determinant of Sequence Recognition	Zinc Finger Proteins	Transcription Activator-Like Effectors	crRNA or sgRNA
Mechanism of Recognition	Protein-DNA	Protein-DNA	RNA-DNA
Nuclease	FokI	FoKI	Cas9
Success Rate	Low (~24%)	High (>99%)	High (~90%)
Average Mutation Rate	Low or variable (~10%)	High (~20%)	High (~20%)
Length of Target Site	18-36 bp	30-40 bp	2bp (total length 23 bp)
Restriction in Target Site	G-rich	Starts with T and ends with A (owing to the heterodimer structure)	Ends with an NGG to NAG (lower activity) sequence
Design Density	One per ~100 bp	At least on per bp	One per 8bp (NGG PAM) or 4 bp (NGG and NAG PAM)
Multiplexing	Rarely Used	Rarely Used	Capable – able to put the RNA guide sequence into more than one target sequence so you can cut simultaneously in many different places than one)
Off-Target Effects	High	Low	Variable
Cytotoxicity	Variable to High	Low	Low
Size of Construct	~1kb x 2	~3kb x 2	4.2kb (Cas9 from streptococci's pyogenes) + 0.1kb (sgRNA)
Ease of Design and Use	Costly, difficult to produce and deliver	Less costly to produce, more difficult to deliver	Affordable, easy to modify and deliver

Genome Editing Relies upon DNA DSB repair.
NHEJ or HR

NHEJ – can result in little deletions since there is cleavage of some nucleotides or there can also be some insertions. If this is in a coding region, you can lose the frame very quickly.

HR – uses a template in order to copy the region that is broken to ensure that it is accurate. It is also usable in genome editing.

If you cut the DNA and the cell a donor DNA sequence, most of the time the cell will use the donor DNA sequence rather than the template and out in the changes. We can change exons, amino acids by including donor DNA.
Multiplexing can result in cutting the DNA at two sites and so are able to take out a piece of DNA or even invert it. It can also happen between two chromosomes, resulting in translocation, recreating some the translocations seen in tumors.