Module-9
Lecture-7

DNA repair mechanisms and cancer

Types of DNA damage

We are going to discuss DNA repair mechanisms that are operative whenever there is a damage to DNA

Before learning about the DNA repair mechanisms it is important to know what kinds of damage occur to DNA

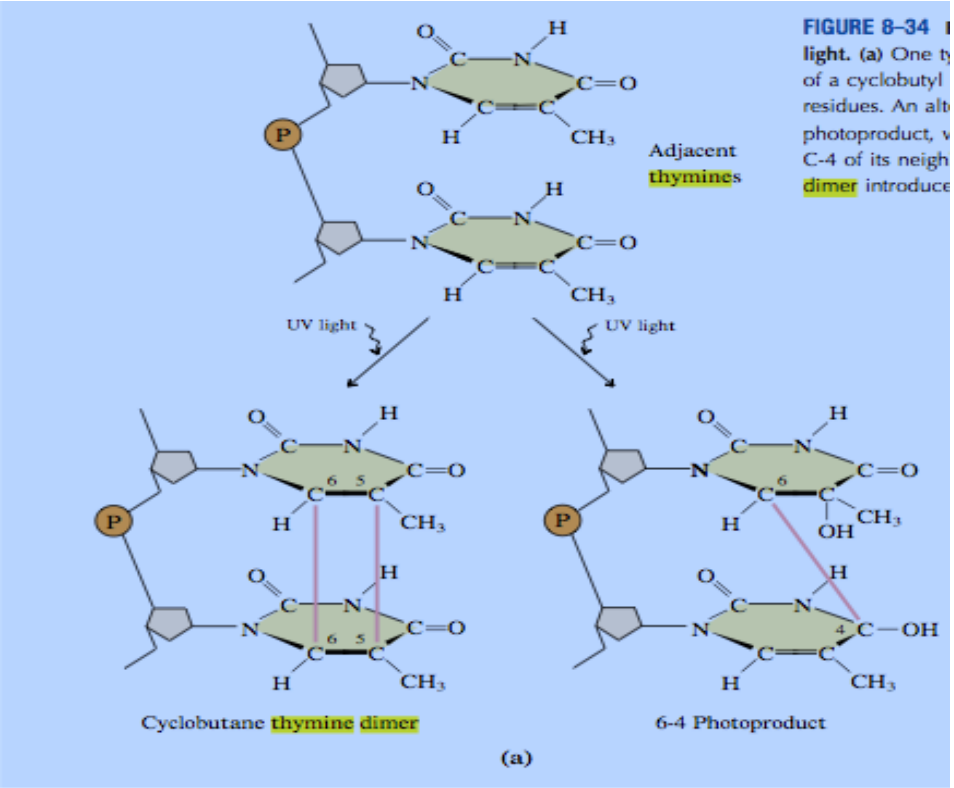
As you know the permanent alterations in DNA structure are called mutations and accumulation of these mutations have various consequences

Spontaneous changes also occur in DNA

For example cytosine residues can undergo spontaneous oxidative deamination to form uracil although this occurs at a slower rate

Hydrolysis of N-beta glycosyl bond between a purine/pyrimidine base and the sugar can occur and this is happening at a relatively higher rate for purines than for pyrimidines. For RNA this is still slower.

Radiation damage



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Environmental agents such as radiation and chemicals also cause DNA damage

UV radiation can damage DNA forming 2 types of thymine dimers and the cyclobutane dimer introduces a bend/kink into DNA.

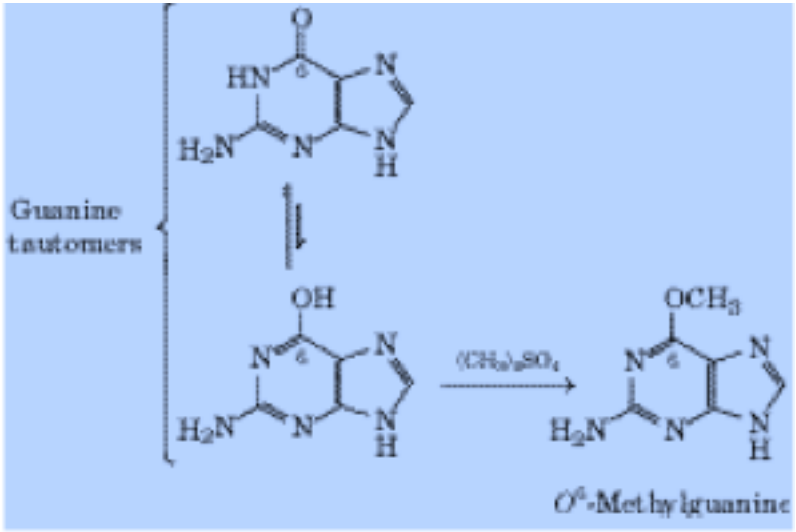
X-rays and Gamma rays can also damage DNA by fragmenting the bases after opening their rings and breaking the covalent links

Chemical damage

DNA can be damaged by chemicals such as nitrous acid (HNO_2) and alkylating agents. HNO_2 is used as a food preservative but does not pose a big threat because of the small quantities used

Alkylating agent such as dimethyl sulfate methylates guanine to form O^6-methyl guanine affecting the base pairing

Hydroxyl radicals can cause DNA damage. Reactive oxygen species formed by our cells are tackled by many enzymes but under some conditions they can still cause damage.



DNA repair - Introduction

Normal DNA replication process itself can generate some errors such as a mismatched base pair

Obviously this can result in mutations such as
Insertion or deletion mutation
Substitution mutation

In a day many thousands of such changes occur in our cells but the DNA repair mechanisms see to that fewer than 1 in 1,000 becomes a mutation and thus making DNA as a relatively stable molecule.

If DNA repair mechanisms are defective the damaging reactions may accumulate and collectively they can aggravate the situation favorable for tumor progression say for example

DNA repair relies on the fact that DNA has two complimentary strands with Watson-Crick base pairs. The advantage of this structure is that if one of the strands is damaged, the other one can act as a template to synthesize the damaged strand

Anticancer agents induce DNA damage

DNA damage is directly induced by many anticancer agents that act as alkylating agents such as
Cyclophosphamide,
1,3-bis[z-chloroethyl]-1-nitrose-urea [BCNU],
Cisplatin, carboplatin, oxaliplatin), and
Adriamycin

Double-strand breaks induced by

Ionizing radiation and bleomycin

Combination of these drugs cause more DNA damage than when a single therapy is used suggesting some synergy with these drugs

What is emerging is a class of chemosensitizers that are merely DNA repair inhibitors

These compounds work better in combination with traditional DNA damaging cytotoxic drugs

DNA repair pathways

DNA repair is basically a cellular response that rectifies DNA damage by restoring the normal base pairs and the original structure

The different pathways of DNA repair are

Base excision repair (BER)

Nucleotide excision repair (NER)

Mismatch repair (MMR)

Homologous recombination (HR)

Nonhomologous end joining (NHEJ)

and translesion DNA synthesis (TLS)

DNA repair pathways

Is there any advantage in having many pathways?

Yes. When there is a small problem with one pathway another pathway can partially compensate

Sometimes if one pathway is completely absent the cells now start depending on another pathway

The DNA repair pathways are highly regulated.

DNA repair pathways are differentially active in various tissues and cell types

Base Excision Repair

This pathway takes care of damaged DNA bases or single-strand DNA breaks

This pathway is useful when there is a spontaneous DNA damage (DNA deamination or hydroxylation of bases) or the damage is by alkylating agents.

DNA glycosylases remove the bases damaged

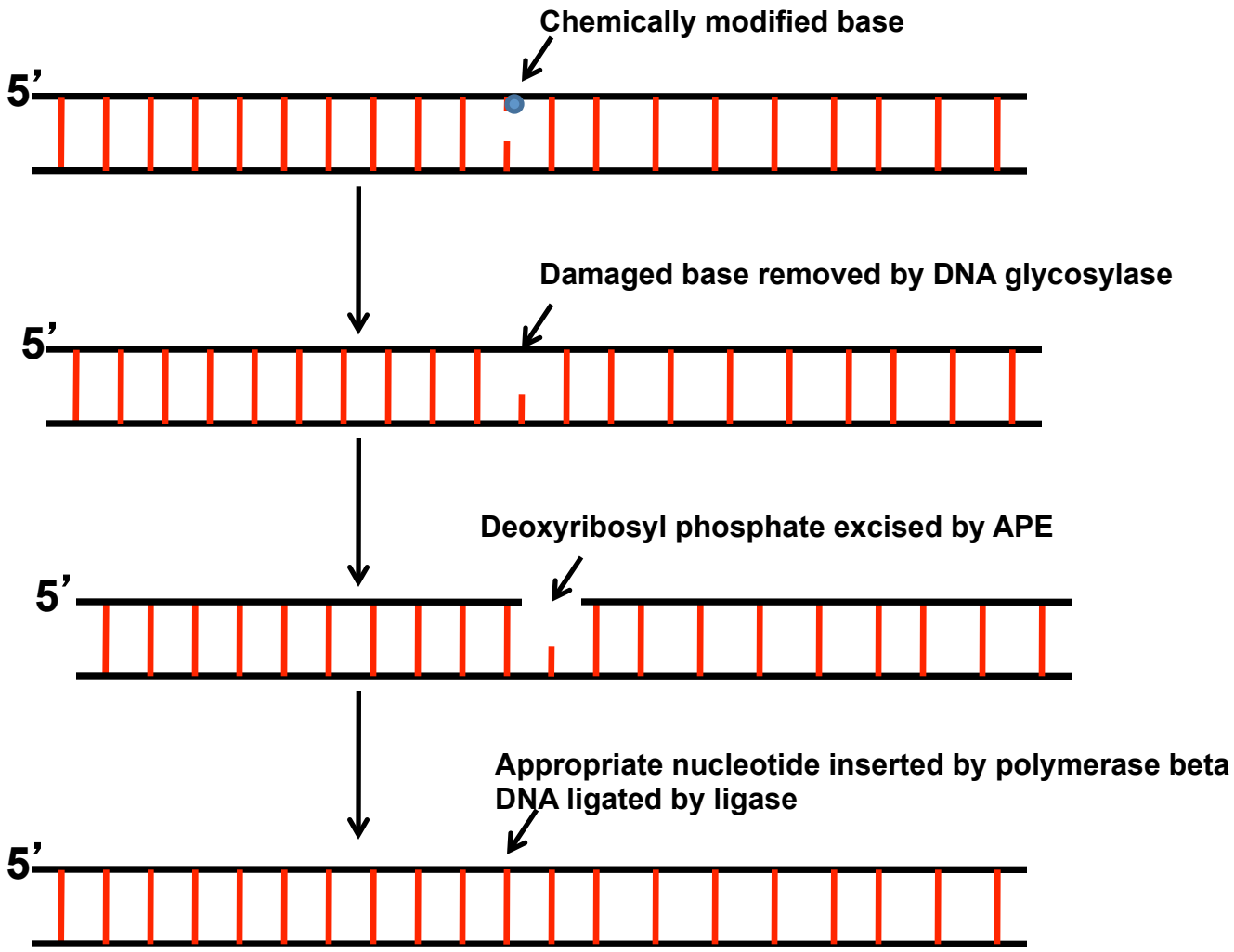
This results in the formation of what is called apurinic or apyrimidinic site (AP site)

Ape1 endonuclease that specifically recognizes sugars without attachment to the bases acts now and forms 5′ deoxyribose-phosphate

DNA polymerase fills up the single strand breaks either with a single nucleotide or with a longer repair patch

This is followed by ligation by a ligase enzyme

Base Excision Repair



Mismatch repair (MMR)

As you know DNA replication is prone to errors and these errors in the form of mismatched nucleotides are corrected by this pathway

It also detects and corrects DNA adducts such as those formed upon treatment with platinum-based chemotherapeutic agents.

Many genes such as *MSH2, MLH1, PMS2, MSH3, MSH6, and MLH3,* are involved in mismatch repair (Mut S homolog; Mut L homolog)

The heterodimeric MSH complex recognizes the nucleotide mismatch

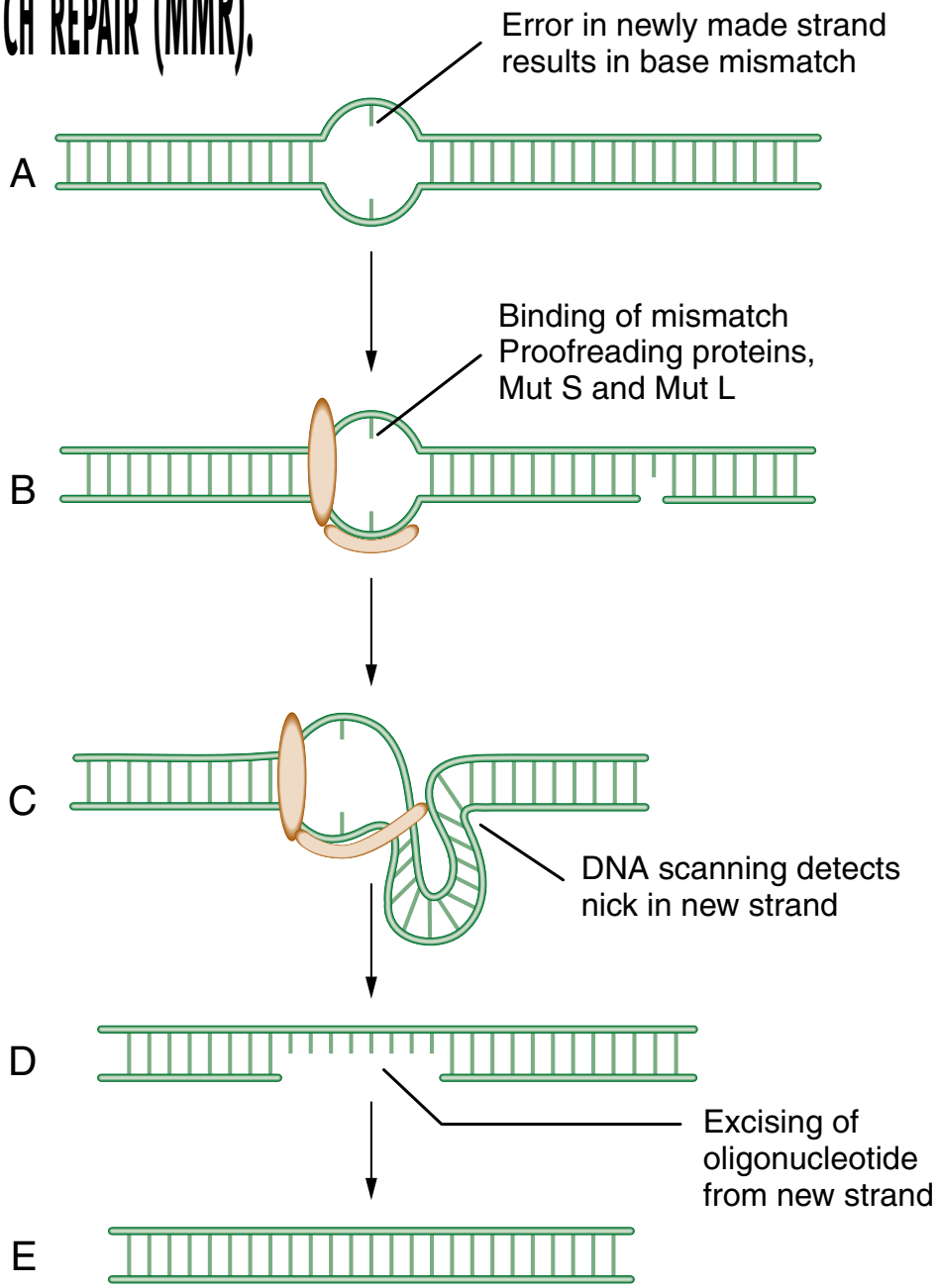
Then it interacts with MLH1/PMS2 and MLH1/MLH3 complexes

Nucleotide excision and resynthesis involves many proteins

If cancer cells are deficient in mismatch repair they will have much higher mutation frequencies than normal cells

Such cancer cells exhibit microsatellite instability

Schematic model of mismatch repair (MMR).



NER pathway

This pathway takes care of DNA adducts

XPA, XPB, XPC, XPD, XPE, and XPG proteins are involved in this pathway

XPF and ERCC1 proteins are required for the processing of DNA cross-link repair.

These proteins cooperate to recognize and excise the damaged nucleotides and resynthesize and ligate the damaged DNA strand.

A DNA-binding component, the DDB initially binds to sites of damaged DNA, such as cyclopyrimidine dimers or 6–4 photoproducts.

DDB is part of a ubiquitin E3 ligase that polyubiquitinates XPC.

Polyubiquitination of XPC results in enhanced DNA binding.

NER pathway

This binding sets the stage for the downstream binding of the entire excision repair complex, TFIIH, thus leading to excision of the damaged bases.

Eukaryotic NER involves

transcription coupled repair (TCR)

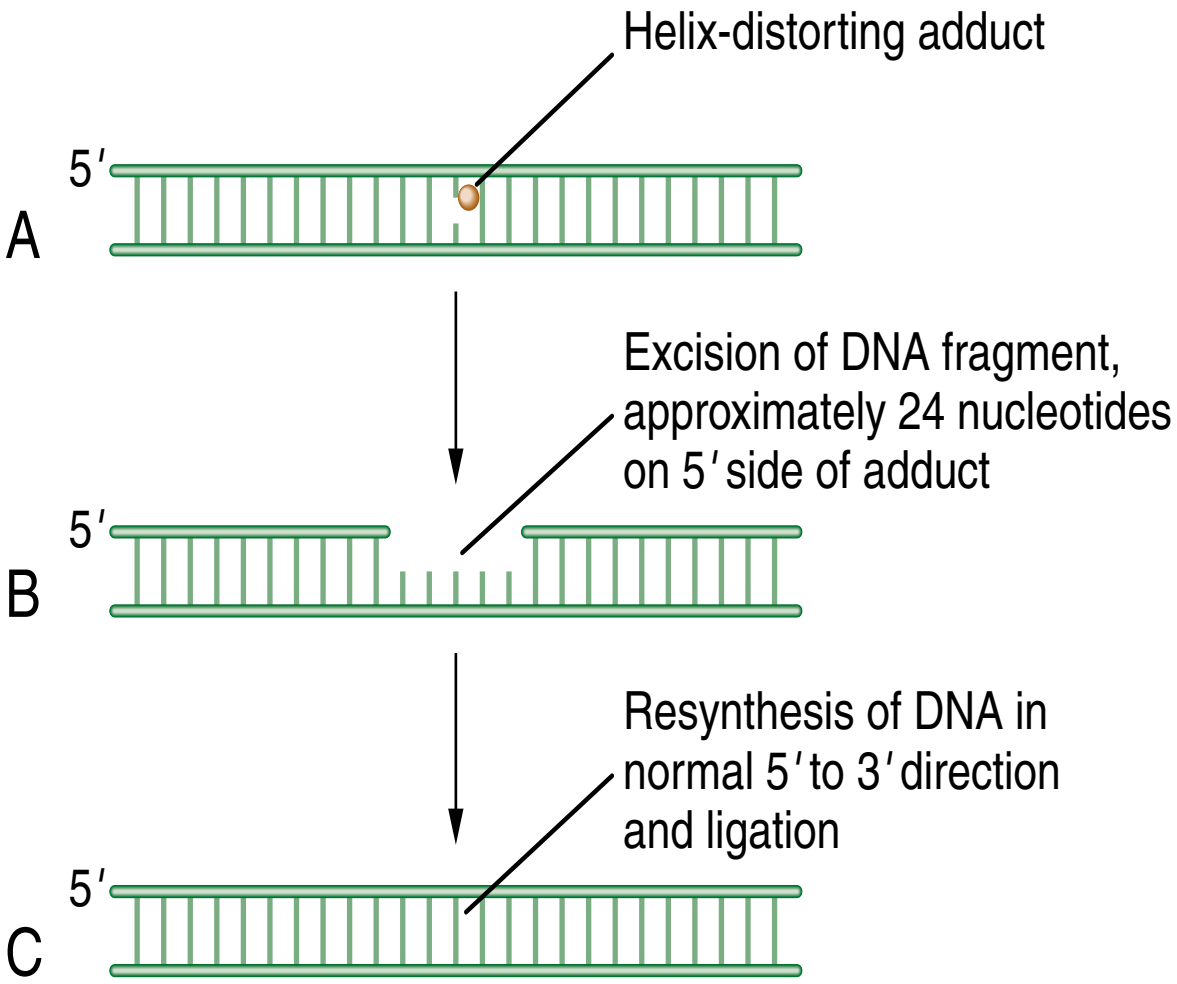
and global genome repair (GGR).

GGR is a slow, random process of inspecting the entire genome for injuries,

whereas TCR is highly specific and efficient and concentrates on damage-blocking RNA polymerase II.

The two mechanisms differ in substrate specificity and recognition, and hence the enzymes involved are important nodal points for post-translational modifications.

Schematic model of nucleotide excision repair (NER).



Homologous Recombination Repair

This takes care of DNA double-strand breaks (DSBs)

DSBs can be caused by reactive oxygen species, Ionizing radiation, bleomycin, anthracyclines, and topoisomerase inhibitors

Normal S-phase progression in cell cycle can also cause DSBs

Failure to repair DSBs results in mutations, gross chromosomal rearrangements and other aberrations, and eventually cell death.

DSBs are repaired through the alignment of homologous sequences of DNA and occurs primarily during the late S to M phase of the cell cycle.

RAD50-MRE11-NBS1 complex, having a 3′–5′ exonuclease activity, exposes the 3′ ends on either side of the DSB, a process that may also require *BRCA1.*

Homologous Recombination Repair

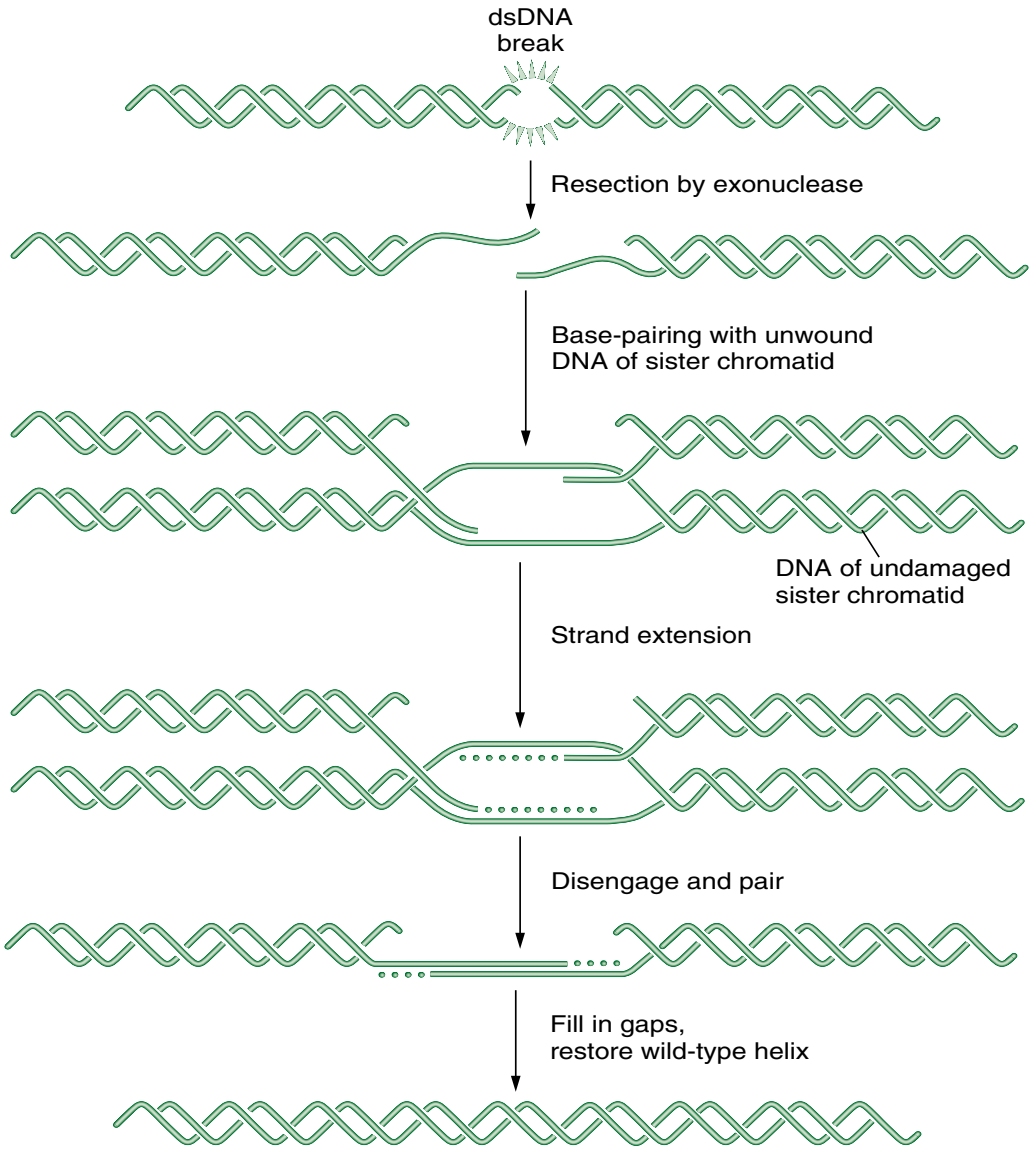
The 3′ advancing strand from the damaged chromosome then base pairs with the complementary sequence of the homologous chromosome

This process requires BRCA2, and RAD51.

The 3′ end of this strand is then extended by an HR polymerase by reading off this complementary sequence.

3′ end of the advancing strand returns to the original chromosome and replication continues.

Schematic representation of homologous recombination



Nonhomologous End Joining

Similar to HR, this pathway is important in the repair of agents that result in DSBs such as Ionizing radiation, bleomycin, topoisomerase II poisons, and anthracyclines.

The DNA-dependent protein kinase (DNA-PK) consists of the catalytic subunit (DNA-PKcs) and the regulatory subunit (the Ku70/Ku80 heterodimer).

The DNA-PKcs subunit is a serine/threonine kinase that belongs to the phosphatidyl inositol-3 kinase family.

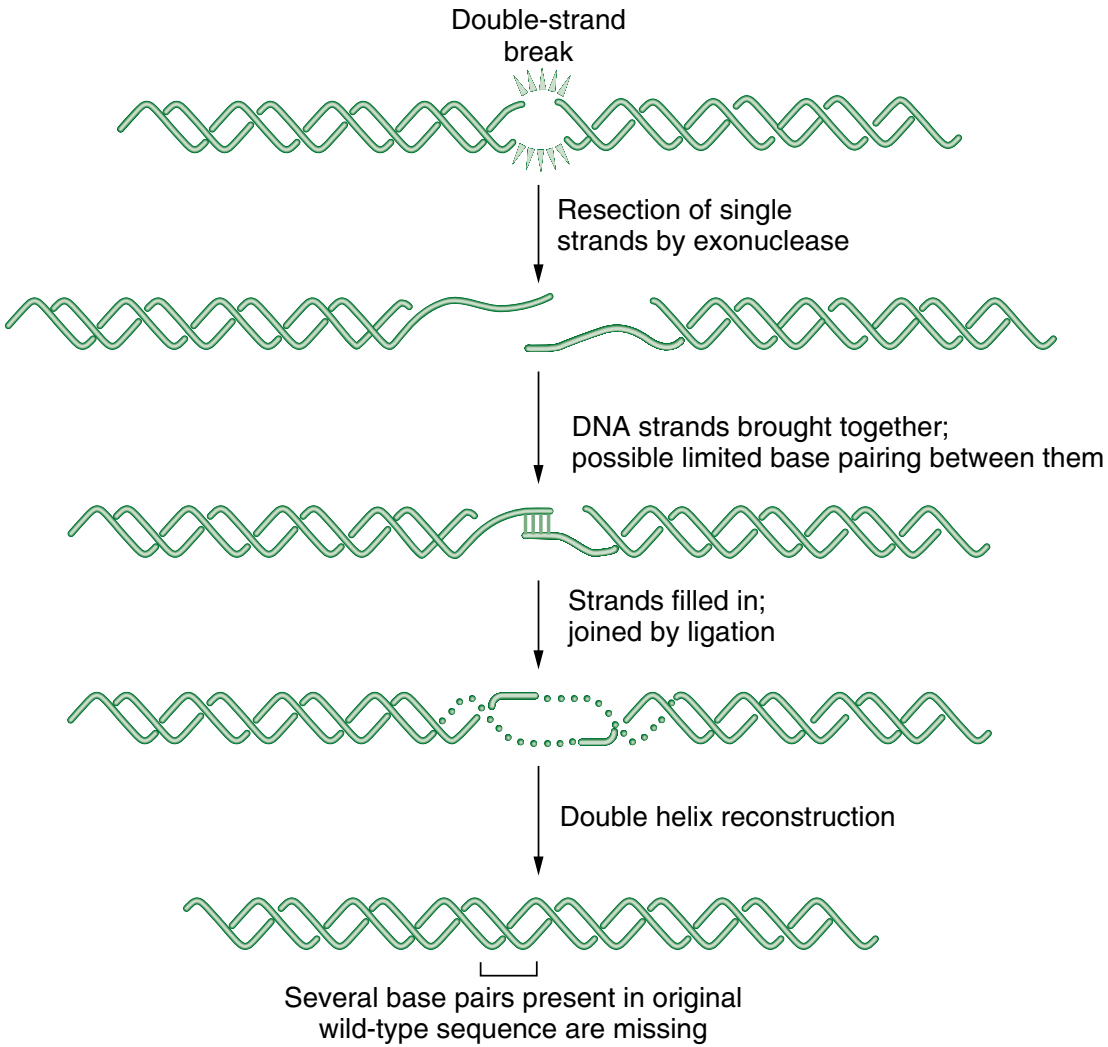
The Ku80/Ku70 heterodimer (Ku) exhibits sequence-independent affinity for double-stranded termini and on binding to DNA, ends recruits and activates the DNA-PKcs catalytic subunit.

Additional proteins are needed such as the artemis protein and DNA ligase IV.

Since the process does not use a complementary template, the fusion of the blunt-ended DNA duplexes may result in deletion or insertion of base pairs

NHEJ has a normal function in immune cells to generate diversity at the immunoglobulin and T-cell receptor gene loci.

Schematic representation of nonhomologous end joining (NHEJ).



Translesion DNA Synthesis

The process of TLS is another mechanism for dealing with thymine dimers and bases with bulky chemical adducts.

At a DNA replication fork, DNA adducts may stop a replicative polymerase, such as DNA polymerase Δ.

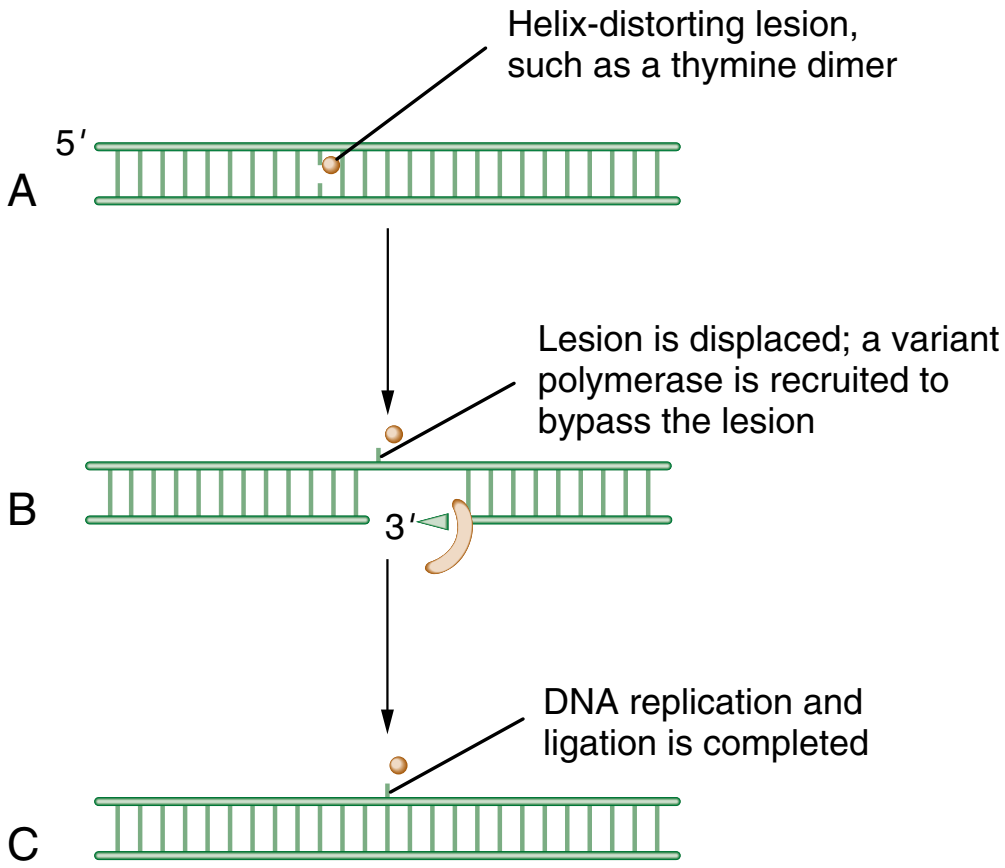
Cells therefore, switch on alternative polymerases

such as Pol eta, which will replicate past certain DNA lesions with high fidelity.

Human cells have at least 15 DNA polymerases and details about their mechanisms are yet to be unraveled

Schematic model of translesion synthesis repair (TLS).

pathway per se, it is a mechanism of DNA damage bypass. In t



DNA Repair Pathways and cancer

Since a specific DNA repair pathway can antagonize the activity of anticancer agents, its status in a tumor may help to predict a suitable therapy for the patient

BER and NER pathways remove the DNA bases modified by monofunctional alkylating agents

MGMT (O^6-methylguanine-DNA methyltransferase) gene promoter methylation in gliomas and colorectal tumors switches off this enzyme and this leads to the hypersensitivity of these tumors to some monofunctional alkylating agents

In addition, damaged bases in the DNA can be bypassed through the use of TLS.

Rapid TLS (damage avoidance) is essential for a cell to go through S phase rapidly, without replication arrest and apoptosis

DNA Repair Pathways and cancer

TLS is an error-prone process, however, and the use of TLS by cancer cells may result in their increased mutation frequency.

Some of the 15 variant polymerases can extend a nascent DNA strand past a thymine dimer or past a bulky DNA lesion.

Other variant polymerases can replace a single nucleotide at the site of an unpaired base.

Pol eta, is mutated in the autosomal recessive human disease, XP-variant (xeroderma pigmentosum variant).

Absence of the Pol eta enzyme results in UV light hypersensitivity, an inability to replicate past thymine dimers, and a predisposition to squamous cell cancers.

Ionizing radiation causes DSBs and oxidative DNA damage.

HR repair and NHEJ repair, can deal with DSB damage in a cancer cell

DNA Repair Pathways and cancer

Tumors that have defects in these DNA repair processes are particularly sensitive to the cytolytic activity of ionizing radiation

Also, radiation resistance can emerge through the induction of these DNA repair activities in treated tumor cells.

Tumor cells that grow in a more hypoxic environment may also be more resistant to the killing effect of ionizing radiation, perhaps due to the decrease in oxidative damage generated in these cells.

Regulation of DNA Repair

The major proteins involved with DNA repair include sensory (DNA binding) proteins, enzymes that remove damaged bases, and enzymes that restore the normal DNA sequence.

Regulatory enzymes, such as helicases, serve to load DNA repair complexes at the sites of DNA damage.

Topoisomerases, serve to unwind damaged DNA to facilitate DNA repair complex assembly, loading into chromatin, and disassembly.

In BER, a sumoylating enzyme modifies one of the glycosylases, TDG, thereby enhancing the activity of the glycosylase in removing damages bases.

In NER, an E3 ligase complex (Cul4A,DDB1, DDB2) activates the polyubiquitination of the XPC protein.

Regulation of DNA Repair

This XPC modification is a necessary event for the downstream activity of the NER complex.

In TLS, an E3 ligase, RAD18, monoubiquitinates the DNA processivity factor, PCNA, and allows this clamp to interact with the downstream DNA polymerase Pol eta.

PP2A removes phosphate from ATM substrates and thereby switches off the DNA damage response.

The deubiquitinating enzyme, USP1, can remove ubiquitin from activated FANCD2 and thereby switch off homologous recombination repair.
USP1 can also deubiquitinate PCNA and switch off TLS repair.

Loss of these regulatory mechanisms may result in the failure to activate an error-free DNA repair pathway or (2) inactivate an error-prone DNA repair pathway.

In either case, the consequence may be a heightened mutation frequency of the dysregulated cell and a predisposition to cancer.

Repairing DNA Cross-Links

Inter strand DNA cross-links (ICLs) involve the covalent modification of both strands of DNA, the lesions can prevent DNA strand separation during DNA replication.

The lesions can also prevent the access of some DNA repair enzymes and transcription factors that normally require DNA strand separation for DNA binding to occur.

DNA cross-linking agents, such as cisplatin derivatives (carboplatin and oxaliplatin) and mitomycin C, are especially cytotoxic to tumor cells, and their therapeutic index derives, at least in part, from the high proliferative rate of tumor cells versus normal cells.

Cross-link repair in human cells probably requires multiple DNA repair pathways.

According to this model, the ICL is only repaired during S-phase progression.

Initially, an advancing replication fork encounters an ICL.

An unknown endonuclease cleaves the DNA, thus generating a DSB.

Next, a second endonuclease is invoked to cleave the DNA after the DNA cross-link.

This endonuclease may be composed of the ERCC1/XPF protein

Repairing DNA Cross-Links

The cross-linked single-strand fragment can be flipped out of the helix.
First, TLS allows bypass of the crosslink and replication and ligation of the upper double helix.
Next, the NER pathway can excise a stretch of damaged DNA and allow gap filling of the excised oligonucleotide.
Finally, HR repair can be used for the error-free, template-driven repair of the damage.
The result of this sequential use of three independent DNA repair pathways is to resume DNA replication and restart the replication fork.

DNA Damage Responses

DNA damage responses include the activation of cell cycle checkpoints, the activation of apoptosis, and the activation of DNA damage tolerance.

In the case of DNA damage tolerance a cell accepts DNA damage and continues DNA replication even when the mutation frequency is high.

These responses require a DNA damage sensor (such as a sensor kinase, ATM or ATR), an effector kinase, and downstream protein machines dedicated to DNA repair, apoptosis, or checkpoint activities.

DNA repair is, thus only a part of the broader DNA damage responses

DNA Damage Responses

DNA adduct or a thymine dimer, can activate a sensor kinase, such as ATM, ATR, or DNA-PK.

These kinases get autophosphorylated and also phosphorylate a large number of substrates.

The ATM kinase is the product of the ATM gene, the gene mutated in the cancer susceptibility disorder, ataxia-telangiectasia.

Activated ATM and ATR proteins phosphorylate additional downstream "effector" kinases, such as the checkpoint kinases, Chk1 and Chk2.

Activated Chk1 and Chk2 phosphorylate a wide array of protein targets involved in the machinery of DNA repair or DNA damage checkpoints.

DNA Damage Responses

In response to ionizing radiation, a DSB is generated, and this activates ATM.

ATM subsequently phosphorylates Chk2, which, in turn, phosphorylates the cell cycle activator, cdc25A.

Cdc25A phosphorylation leads to its rapid degradation and a cell cycle arrest.

This appears to be an important mechanism by which a cell can respond to DNA damage: by arresting its cell cycle progression in S phase.

This mechanism makes a cell to slow down the cell cycle and gets some time to repair its DNA or, it may undergo apoptosis if the damage is severe.

In ATM-deficient cells, S-phase progression continues even with DNA damage.

The cell will then have a higher mutation rate and end with mitotic catastrophe

This phenotype is known as radioresistant DNA synthesis (RDS), and it is the hallmark of a cell with a defect in the ATM-Chk2-cdc25A axis.

DNA Damage Responses

DNA damage response leads to the assembly of proteins in subnuclear foci (ionizing radiation inducible foci).

Multiple ATM- and ATR-phosphorylated substrates, such as Chk1, BRCA1, and BARD1, assemble in foci following DNA damage.

Phosphorylated BACH1 can bind directly to the BRCT domain (a phosphoserine receptor) of the BRCA1 protein.

The number of foci correlates with the number of unprocessed double-strand DNA breaks, and the foci are widely believed to be sites of DSB repair.

pATM, pBRCA1, and pFANCD2 colocalize in Ionization radiation induced foci.

DNA Damage Responses

Phosphorylated RAD51 also assembles in foci during normal S-phase progression.

These "replication foci" are believed to be sites of DNA repair by HR between sister chromatids, which occurs during normal DNA replication.

cells that are defective in the formation of DNA repair foci are themselves defective in DNA repair.

Cells deficient in RAD51 foci are defective in HR repair and are hypersensitive to IR.

Cells defective in the assembly of polyADP ribose (PAR) foci are defective in the repair of single-strand breaks and may therefore have an underlying defect in BER.

As such, tumor cells missing particular types of DNA repair foci may be more sensitive to certain kinds of chemotherapy or radiation.

DNA Damage Responses

Human cancers are often deficient in the DNA damage response.

Germ-line mutations in DNA damage response genes, such as ATM, NBS1, FANCD2, BRCA1, and BRCA2, can result in an increased susceptibility to cancer.

Individuals who inherit a single mutant allele of BRCA1, have a high risk of developing a breast, ovarian, or prostate cancer during their lifetime.

The tumor results from the inactivation of the second BRCA1 allele through deletion and loss of heterozygosity, thus resulting in a tumor with a specific DNA repair defect.

BRCA (−/−) tumors therefore have genomic instability, but also have increased sensitivity to some DNA-damaging agents such as ionizing radiation and DNA cross-linkers.

DNA Damage Responses

ATR and CHK1 are activated at the advancing replication fork, leading to the activation of HR repair.

NHEJ is hyperactive in nondividing cells and functions as the major mechanism of DSB repair in these cells.

DNA damage response, as in the inherited disease, ataxia-telangiectasia, may lead to a characteristic constellation of clinical findings, including cerebella degeneration and lymphoma predisposition.

A somatic disruption of the same pathway (e.g., the ATM CHk2-p53) may lead to a very different set of cancers, such as the solid tumors of bladder and ovary.

As cells progress from the premalignant state to the malignant state, they lose these DNA damage responses, perhaps through acquired disruptions of ATM or CHK2 activity

Inherited Chromosome Instability Syndromes

HR and TLS repair is defective in Fanconi anemia cells.

NER repair is defective in xeroderma pigmentosum cells, Cockayne syndrome cells, and trichothiodystrophy cells.

MMR repair is defective in children with Turcot syndrome and in tumor cells derived from adult patients with hereditary nonpolyosis colorectal cancer (HNPCC).

TLS repair is defective in patients with XP-variant disease. Most of these pediatric diseases exhibit autosomal recessive inheritance, such as XP, Fanconi anemia (FA), and Cockayne syndrome (CS).

Turcot syndrome has been reported to exhibit autosomal dominant or autosomal recessive inheritance depending on the particular mutation affecting MMR.

Inherited Germ-Line DNA Repair Deficiency

Patients with inherited DNA repair deficiency syndromes are prone to the development of specific tumors.

Patients with FA, for example, are predisposed to acute myeloid leukemia and squamous cell carcinomas, primarily of the head and neck or gynecologic sys- tem.

Patients with XP are prone to skin squamous cell carcinomas, primarily on body surfaces with more sunlight exposure.

Patients with HNPCC and an inherited MMR deficiency are prone to colon cancer and ovarian cancer.

A germ-line mutation in the retinoblastoma (Rb) gene may result in an embry- onal tumor, such as a retinoblastoma or a pineoblastoma, but a somatic disruption of the Rb gene may lead to the development to of a sarcoma.

Somatic disruptions

Somatic disruption of the FA pathway results in a wide range of tumor types, including tumors of the ovary, lung, and cervix.
Moreover, somatic disruptions result from methylation and silencing of an upstream FA gene (FANCF).
Germ-line disruption of the same genes results from inherited mutations, such as missense mutations or nonsense mutations.
Somatic disruption of the NER pathway plays a role in the development of testicular cancer and appears to account for the hypersensitivity of this tumor to the drug, cisplatin.
Paradoxically, somatic disruption of a DNA repair pathway can also result in chemotherapy resistance.
Studies indicate that methylation and silencing of the MLH1 gene may account, at least in part, for the cisplatin resistance of some ovarian tumors.
Disruptions of the other DNA repair pathways have been observed in sporadic human tumors, accounting, at least in part, for the specific drug- and radiation-sensitivity spectrum of these tumors and their clinical outcome.
HR is disrupted in breast and ovarian cancer, NER is disrupted in testicular cancer, and MMR is disrupted in sporadic colon cancer.

DNA Repair Pathways and Mutation Rates

The increased mutation rate results in large part through the disruption of DNA repair pathways.

The MMR pathway normally functions to improve the fidelity of DNA replication by quickly identifying and excising mismatched bases generated by faulty DNA replication.

Loss of the MMR pathway by germ-line mutation or somatic mutation, can lead to a “mutator” phenotype.

This phenotype can be readily detected by microsatellite instability in the genome of the cancer cell.

DNA Repair Pathways and Mutation Rates

This increase in mutation rate can also be accounted for by an increase in error-prone DNA repair mechanisms.

In the setting of elevated translesion synthesis, some error-prone polymerases, such as Rev3, may increase the frequency of point mutations in the genome of the human cancer.

Also, an elevation in the error-prone NHEJ pathway may account for the elevated complex mutations (insertions and deletions), observed in some cancers.

Many human tumors have been found to express abnormal levels of polymerase b, which may also contribute to their increased mutation frequency.

Defects in the NER pathway may account for cisplatin sensitivity of some testicular and non-small cell lung cancers

Defects in HR repair may account for cisplatin sensitivity of ovarian and head and neck carcinomas.

Predicting Chemotherapy Responsiveness

NER pathway appears to account, at least in part, for the cisplatin hypersensitivity of testicular cancers

Deregulation of NER pathway may be due to frame shift or nonsense mutations in NER genes or from epigenetic changes, such as methylation and silencing of NER genes.

This may also be due to DNA repair gene polymorphisms in the germ-line of the cancer patient.

Common SNPs are known for the NER genes XPD, ERCC1, and XRCC1.

SNPs in multiple NER genes appear to account, at least in part, for the cisplatin hypersensitivity of some squamous cell carcinomas and lung cancers.

Whether these SNPs will serve as predictive biomarkers for chemotherapy or radiation sensitivity is an open question now.

Summary

We discussed as to how the cells would acquire DNA damage spontaneously as well as through other factors/agents

We learnt about the DNA repair mechanisms specifically the six major DNA repair pathways BER, NER, MMR, HR, NHEJ, TLS

We learnt about the regulation of DNA repair and discussed repairing of DNA cross links that occur under certain conditions

We discussed the relationship between DNA repair and cancer

DNA damage responses and cancer

Failure of DNA repair and the diseases such as FA and XP

DNA repair and the mutation rates

Predicting the chemotherapy responses in connection with DNA repair

END