DNA damage repair in cancer therapy

Mutations in repair pathways

Ingrid de Jong

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Abstract

Cells have two systems essential for maintaining the genome integrity of a cell population: DNA repair and apoptosis. Mutations in each of these systems can cause cancer. This literature thesis focuses on aberrations in the DNA damage repair, that causes an increase in the risk of cancer. In single strand break (SSB) repair, the major difference between the pathways, is the type of damage that is recognized, and how it is recognized. However, in DSB repair the major differentiate is in their fidelity of repair. homologous recombination (HR) uses a (nearly) identical template to repair DSBs, while non-homologous end-joining (NHEJ) anneals lose ends without the use of a template. NHEJ is therefore a error-prone pathway. In each repair pathway can occur mutations which goes along with loss of function of a repair pathway. When a repair pathway is heterozygous mutated in healthy cells and homozygous mutated, inducing loss of function, in tumor cells, it is possible to injure cancer cells to a greater extent than healthy cells. The loss of function from one repair pathway in de tumor, can result in an increased cytotoxic effect of DNA damage to cancer cells. However, the cytotoxic effect of DNA damage can be enlarged by inhibition of a second repair pathway. By inhibiting a SSB repair machinery, the remaining SSB are converted to DSB after replication. In cancer known to have loss of function mutations in DSB repair, cancer therapy is possible by inhibition of a SSB repair. Consequently, the damages can be repaired in DSB repair in healthy cells, while tumor cells accumulate DSBs. The use of such targeted therapy can be combined with DNA damage inducing agents like chemo- or radiotherapy, in which success of the therapy depends on whether or not the cancer cell survive the therapy. By disengaging a large part of the DNA repair, cancer cells will be unable to restore the acquired damage and go into apoptosis. This literature thesis will discuss mutations that are known to impair DNA repair, especially mutations in the HR repair pathway.

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Introduction

Cancer is mostly treated with surgery, radiotherapy, chemotherapy and targeted therapy. Surgery offers the greatest chance for cure in absence of metastases. It is often combined with other therapies like radiotherapy or chemotherapy. Radiotherapy damages local cells, while chemotherapy travels through the blood damaging cells all over the body. Both radio- and chemotherapy focus on damaging DNA: chemotherapy by chemicals, and radiotherapy by ionizing radiation such as γ-rays and x-rays. When DNA is damaged, a DNA damage checkpoint recognizes it and induces cell cycle arrest, followed by activation of DNA damage repair mechanisms or cell death. In healthy cells DNA damage repair is necessary to prevent structural damage that alters or eliminates the cell's ability to transcribe the affected DNA causing the formation of dysfunctional or even cancerous cells.

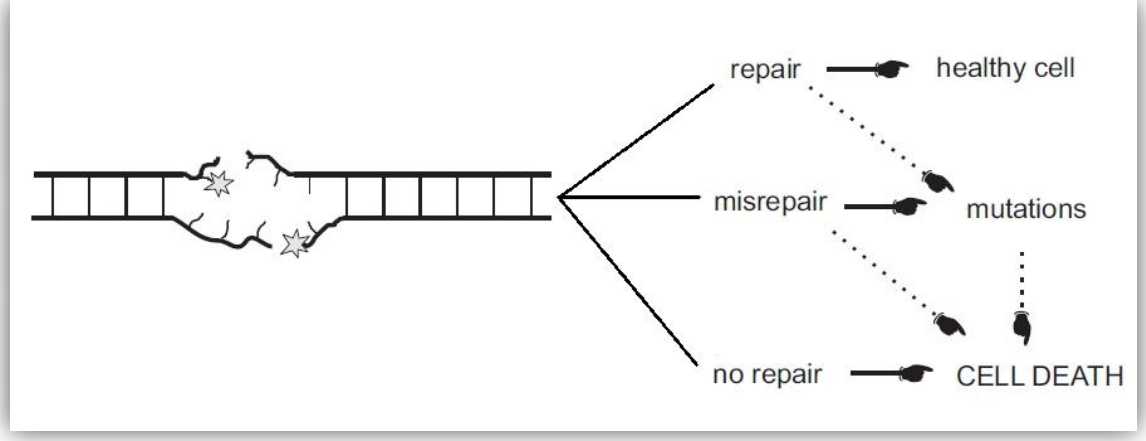


Figure 1. If DNA damage occurs, the damage can be repaired misrepaired or notrepaired ,resulting in healthy cells, mutations or apoptosis.

The aim of both chemo- and radiotherapy is damaging the DNA of cancer cells so badly, that their rapid cell division stops and at his best is followed by apoptosis. However, the DNA damage repair mechanisms can help the cancer cells to survive the acquired damages, making the chemo- or radiotherapy less effective. Therefore, the success of radiation or chemo's is highly restricted to what extent cancer cells can repair their DNA damage, which determines whether or not the cancer cells will survive the therapy. This knowledge brought researchers to focus on increasing the cytotoxic effect of DNA damage inducing agents by inhibition of DNA repair mechanisms. This would increase effectiveness of the therapy, but also intensifies side-effects, therefore, the focus is on silencing the DNA damage repair solely in cancer cells. This literature thesis will focus on cancers with mutations at a DNA damage repair mechanism, making it a potential target for inhibition of a second repair pathway. By impairing a DNA damage repair pathway, besides the already non-functional repair pathway in the cancer cells, cancer cells become unable to repair their damage, while healthy cells still have the ability to repair their damage.[1] An example of this strategy is the use of a PARP-1 inhibitor for patients with mutations in the breast cancer genes BRCA-1 and BRCA-2.[2,3]

DNA Repair defects in tumors

Two systems are essential for maintaining the genome integrity of a cell population, DNA repair and apoptosis. If cells experience DNA damage, repair mechanisms are activated, however, when the DNA damage is too severe, this may lead to programmed cell death. Cells that have defects in DNA repair, accumulate excess DNA damage, likely leading to apoptosis. Cells defective in apoptosis, survive with excess DNA damage and continue replicating DNA, passing mutations on to subsequent cell divisions. These mutations may contribute to the formation of a cancer cell, especially mutations in genes involved in cell division. Consequently, regulation of DNA repair and apoptosis pathways can determine whether a cancer lives and proliferates or dies, making these pathways important potential targets for therapy. The center of attention in this thesis is at the DNA repair machinery.

There are several pathways to repair DNA damage. When the damage is restricted to a single strand, a cell has three possible pathways to repair the damage: Base-Excision Repair (BER), Nucleotide Excision Repair (NER) and MisMatch Repair (MMR). BER corrects non-bulky damage to bases caused by deamination, oxidation and alkylation and also repairs Single Strand BreakS (SSBs). NER repairs damages mostly in the form of pyrimidine dimers and 6-4-photoproducts caused by exogenous agents (such as mutagenic and carcinogenic chemicals and photoproducts generated by sunlight exposure). Unlike BER, that can only correct damaged bases recognized by DNA glycosylase, NER recognizes bulky distortions in the structure of the DNA duplex. MMR machinery recognizes and repairs mismatched nucleotides and insertion/deletion loops, arisen during errors in DNA replication and recombination.

Double Strand BreakS (DSBs) can lead to genome rearrangements. Two main mechanisms for DSBs repair are: Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ). HR requires the presence of an (almost) identical sequence that can be used as a template, and therefore, predominates in the S and G_2 phases, when sister chromatids are readily available. NHEJ ligates broken ends without the use of a template and may therefore introduce minor sequence alterations at the DNA ends. DSBs in G_1 phase are predominantly repaired by NHEJ, thereafter, NHEJ is partially downregulated.

Certain cancers are known to have a mutation on a gene coding for DNA damage recognition/repair and therefore, are potential targets for therapy by DNA recognition or repair inhibition. Patients with an impairment of a DNA damage repair pathway exclusively in the cancer cells, can be medicated with a DNA damage repair inhibitor and, if needed, accompanied by a damage inducing agent. By silencing the DNA repair solely in cancer cells, the accumulation of DNA damage is expected to cause the cancer cells to go into apoptosis, while healthy cells can repair themselves. An additional advantage could be a decrease in side-effects compared to chemotherapy. This chapter will discuss proteins involved in the different repair pathways and cancers know to have mutations that cause inhibition of these pathways.

Base-Excision Repair

BER is initiated by DNA glycosylases, which recognize and remove specific damaged or inappropriate bases, forming APurinic / APyrimidinic (AP) sites. Examples of DNA glycosylases are Ogg1 (recognizes

8-oxoguanine), Mag1 (recognizes 3-methyladenine), and UNG (removes uracil from DNA). AP endonuclease APE(X)1 (or REF1) is next responsible for removal of damaged base(s) of the DNA strand. PolyADP Ribose Polymerase (PARP) binds to the free DNA ends and protects them against degradation, stimulates chromatin relaxation to make repair possible and activates DNA polymerase. The resulting single-strand break can then be processed by either short-patch or long-patch BER. The short-patch BER pathway leads to a repair of a single nucleotide added by DNA polymerase β, in the long-patch BER pathway DNA polymerase β/δ produces a repair of at least two nucleotides. Long-patch BER is supposed to incorporate the first nucleotide by DNA polymerase ß, while the rest is integrated by DNA polymerases δ/ε.[4] Endonuclease FEN1 removes the flap generated during long patch BER. DNA ligase III along with its cofactor XRCC1 catalyzes the ligation step in short patch BER, as DNA ligase I ligates the break in long patch BER.[5,6] Proteins involved are shown in Figure 2.

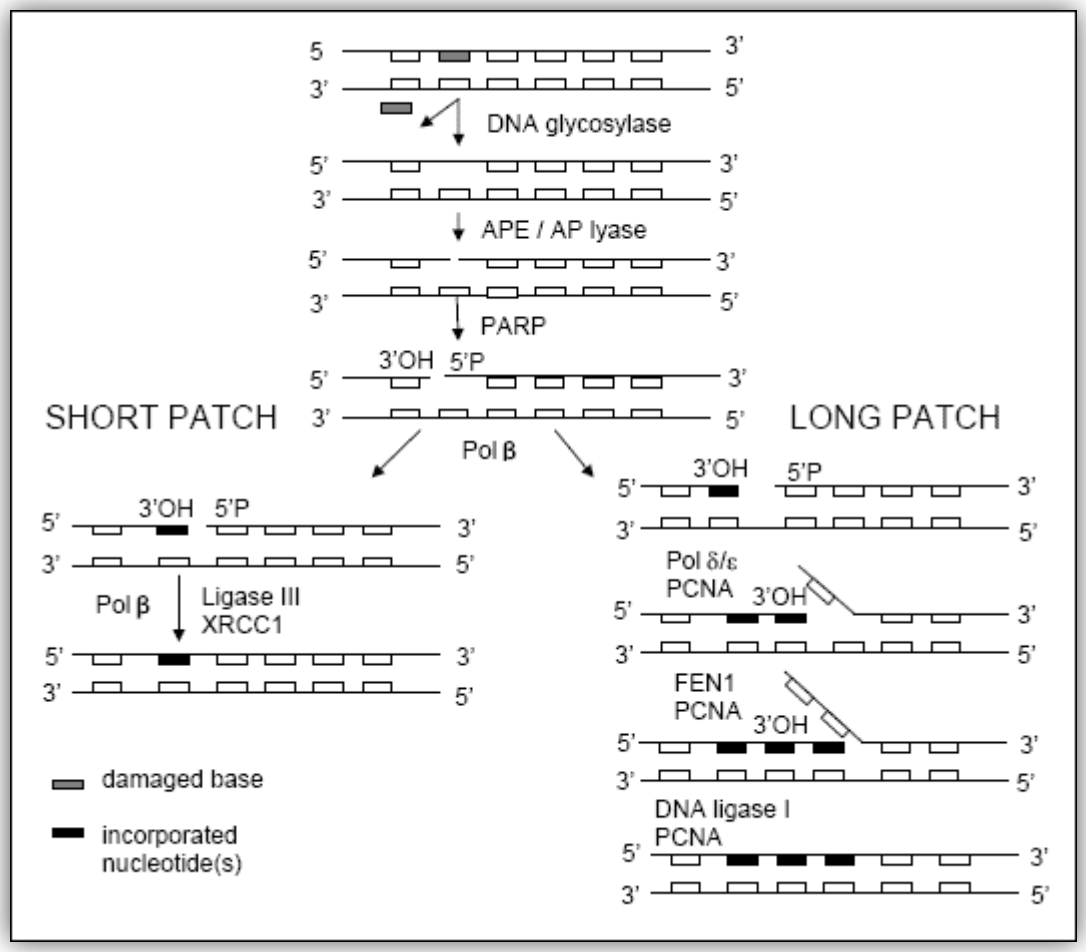


Figure 2. Short- and long-patch pathway of BER. J Tudek B, Swoboda M, Kowalczyk P, Oliński R. Modulation of oxidative DNA damage repair by the diet, inflammation and neoplastic transformation. Physiol Pharmacol. 2006 Nov;57 Suppl 7:33-49.

Mutations are demonstrated in the XRCC1 gene in sporadic breast cancer[7] and alterations in the gene encoding DNA polymerase β (pol β) are identified in colorectal, prostate, lung, bladder, esophageal, gastric and breast carcinomas and in mouse lymphomas.[8,9] The frequent detection of somatic polymerase β alterations in human cancers implies that modification of the polymerase β gene may be correlated to cancer.

Nucleotide Excision Repair

NER can be divided into two subpathways: Global genome NER (GG-NER) and Transcription Coupled NER (TC-NER), that differ only in their recognition of helix-distorting DNA damage. Recognition of GG-NER is mediated by DNA damage binding protein XPC (XPC-hHR23B complex) and XPE complex (DDB1 and DDB2), while TC-NER is activated when the lesion is blocking DNA polymerase II. Both induce the removal of the short single-strand that contains the lesion.

Damage recognition is followed by binding of TFIIH (XPD, XPB, p62, p52, p44, p34 and TTDA complex) through interaction with either XPC or the arrested transcription machinery. TFIIH activates RNA polymerase II transcription and NER.[10] The DNA helicase XPD (ERCC2) unwinds the DNA while XPB binds to the damaged site. XPB and XPC interact with XPD and XPG (ERCC5), facilitating binding to and cleaving of the DNA around the damaged site by the XPG nuclease and the XPF–ERCC1 nuclease. The XPA–RPA complex marks and stabilizes the cleavage sites. At the end, this SSB is filled in by DNA polymerases δ, ε or κ, which uses the undamaged strand as a template. In quiescent cells, ligation involves XRCC1 and ligase III. In proliferating cells, ligation involves ligase I.[11]

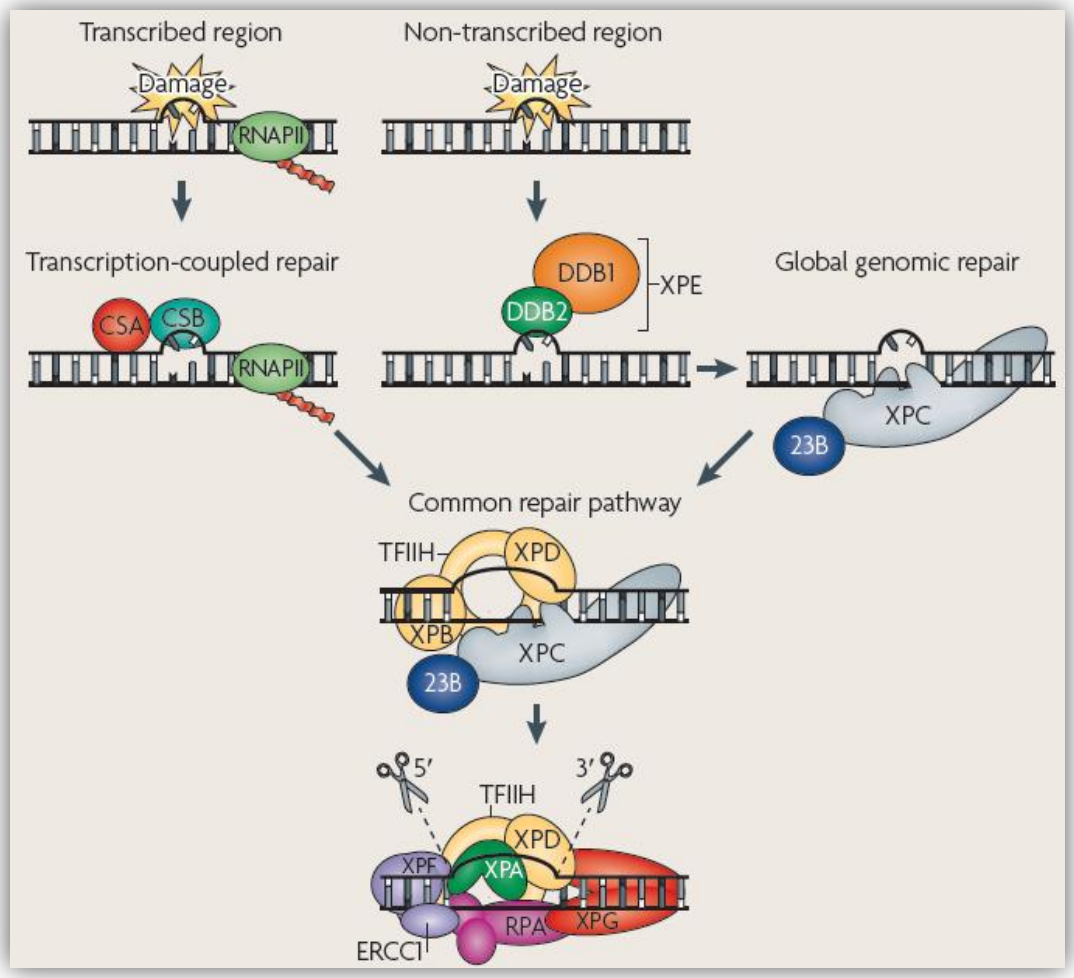


Figure 3. Proteins invoved in Nucleotide excision repair.

Mutations in genes on the nucleotide excision repair pathway are associated with the rare diseases xeroderma pigmentosum, Cockayne syndrome and Trichothiodystrophy, that are characterized by

skin cancer, squamous cell carcinoma, progressive mental retardation, photosensitivity and growth defect. In these diseases genes mutated are respectively, XP-A to XP-G, and XP-V (encoding for DNA polymerase η); CSA, CSB, XP-B, XP-D and XP-G; XPB, XPD, TTDN1 (encodes for C7ORF11, unknown function) and TTDA (TFIIH subunit). [12,13,14]

MisMatch Repair

MMR machinery recognizes base-base mismatches and single-base insertion/deletion loops predominantly by MutSα (MSH2/MSH6 complex). Larger insertion/deletion loops are usually recognized by MutSβ (MSH2/MSH3 complex). DNA glycosylases can convert a base lesion into a single strand break without the need for an AP endonuclease (unlike BER). MLH1 forms a heterodimer with PMS2, PMS1 or MLH3 (MutLα, MutLβ, or MutLγ, respectively). MutLα is a DNA endonuclease that introduces strand breaks in DNA upon activation by MutSα and PCNA. Roles played by MutLβ and MutLγ in mismatch repair are less well understood.[15]

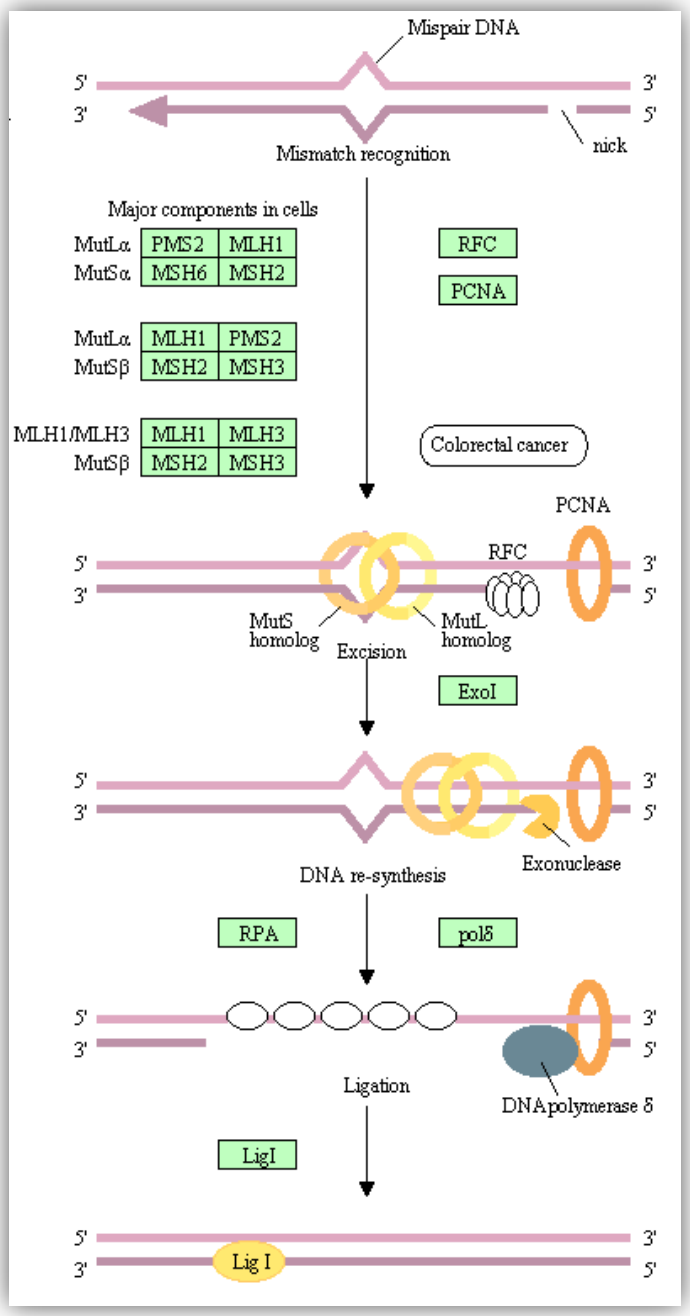


Figure 4. Pathway of mismatch repair.

Mutations in the Mut proteins affect genomic stability. Lynch syndrome (hereditary non-polyposis colorectal cancer) is caused by heterozygous germ line mutations in one of the mismatch repair genes MLH1, MSH2, MSH6, and PMS2. Patients with this syndrome have a predisposition to colorectal, endometrial, and a spectrum of other cancers.[16] MMR defects are also implicated in a number of sporadic tumors. Mutations in MLH1, MSH2, MSH3, MSH6, PMS2, PMS3, MUTYH, OGG1 and MTH1 have been demonstrated in sporadic tumors of the colon, endometrium, stomach, head and neck, cervix, prostate and breast.[17] A subtype of HNPCC is known as Muir-Torre syndrome which is associated with skin tumors, genes affected are MLH1 and MSH2.

Homologous recombination

In HR the sequences are exchanged between two comparable or identical strands of DNA. HR can be subdivided in the DSB repair (DSBR) and synthesis-dependent strand annealing (SDSA). The pathways are alienated after the formation of one Holliday junction, when the second strand has to be synthesized and ligated. In DSBR the second DNA end forms another Holliday junction, while in SDSA the invading strand is passed on and anneals with the resected second end. Both pathways are activated after a double-strand break occurs. The DSBs are recognized by the MRN complex (composed of Mre11, Rad50 and Nbs1 proteins), which unwinds the DNA ends and recruits ATM protein to the site of the break.[18] First Holliday junction is formed by the strand exchange activity of RAD51. The junctions are cut during meiosis resulting in chromosomal crossover or non-crossover.

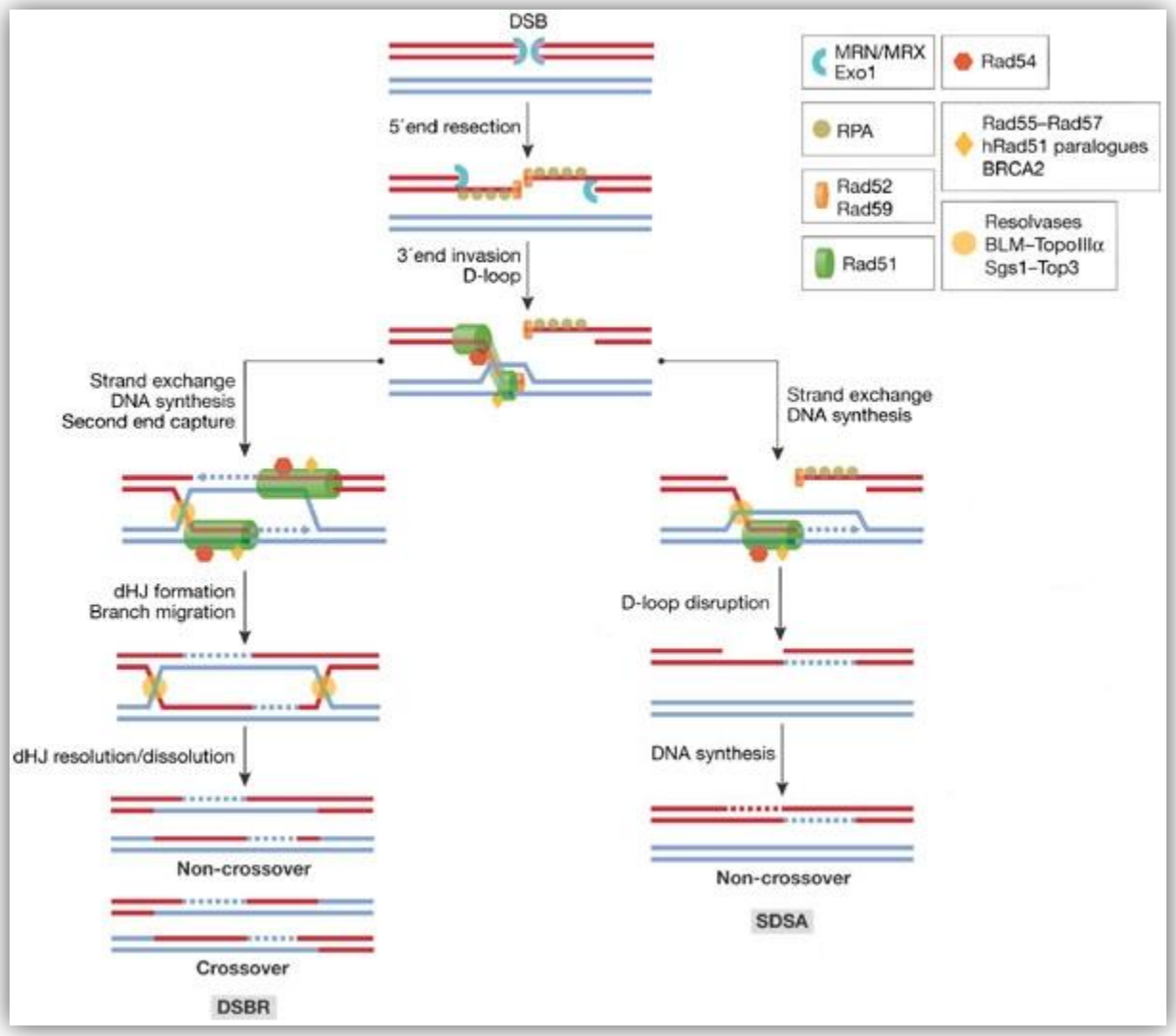


Figure 5. Homologous repair can be subdivided into Double Strand Break Repair (DSBR) and Synthesis-Dependent Strand Annealing (SDSA).

A mutation in the HR pathway is in the NBS1 gene (NBN), which encodes the NBS1 component of the complex MRE11/RAD50/NBS1, resulting in the Nijmegen Breakage Syndrome. Patients with this mutation are characterized by radiation sensitivity and a strong predisposition to lymphoid malignancy. Various mutations in the NBN gene increases the risk of malignant melanoma, stomach

and colon cancer, breast cancer, larynx cancer, and prostate cancer.[19] Another well known mutation is in the BRCA1 and BRCA2 (FANCD1) genes. This mutation is further discussed in the next chapter.

Non-homologous end-joining

NHEJ is referred to as "non-homologous" because the break ends are directly ligated without the need for a homologous template, in contrast to homologous recombination, which requires a homologous sequence to guide repair. A complex of Ku70 (from the XRCC6 gene) and Ku 80 (from the XRCC5 gene) binds the ends of the DSB and allows the recruitment of DNA-PKcs binding to Artemis. Artemis possesses exonuclease activity and interacts with DNA-PKcs providing a mechanism whereby it may be recruited to the DSB. The two DNA-PK molecules interact, causing autophosphorylation of DNA-PKcs. PNK interacts with XRCC4 (X4), thereby promotes phosphate replacement at damaged termini.[20] DNA polymerases μ and λ are recruited to DSBs by interactions with Ku and the X4–L4. Finally, ligation of the remaining nicks is carried out by DNA ligase IV (L4), which exists in complex with XRCC4.[21,22]

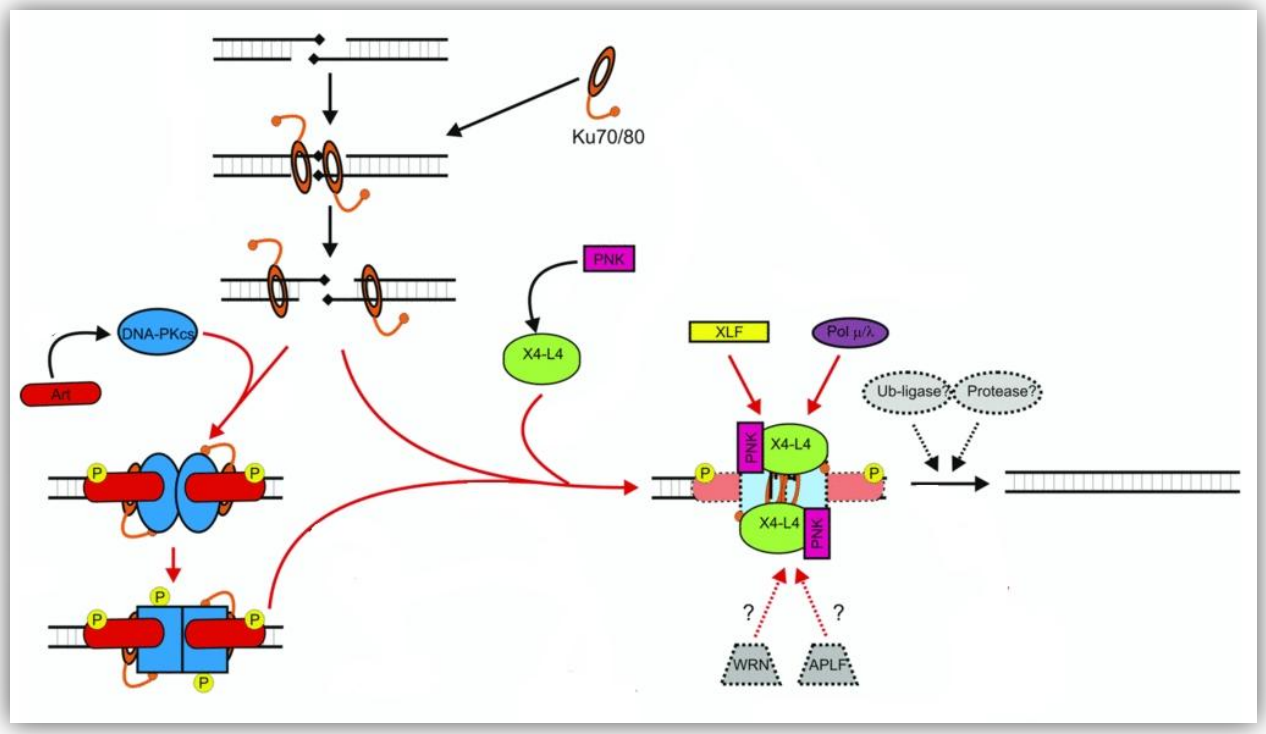


Figure 6. Non-homologous end-joining. Proteins surrounded with dots are suggested to be involved with NHEJ.

Deficiencies in NHEJ that cause cancer are less forthcoming than tumor initiating defects in other DNA repair mechanisms. Deficiency in NHEJ components that cause tumor development has been demonstrated in a *p53* mutated mice for XRCC4, DNA ligase IV, Ku80, and DNA-PKcs.[23] However, loss of p53 in these studies may have masked other deficient pathways involved. In addition, because of the important role, mutation of ligase IV seemed a potential tumor developing deficiency. Patients with the ligation IV deficiency syndrome show besides tumor formation, immunodeficiency and growth retardation.[24,25]

Taking together

Previous pages depict that mutations involved in DNA repair mechanisms, in many cases, can lead to the formation of tumors. Once a recessive mutation is heterozygote present in every cell and during errors in cell's DNA replication or recombination the second allele is damaged to, a repair pathway can be silenced in the cell. When this mutation is undetected and the cell proliferates, a tumor is on his way. Because the tumor is genetically not identical to healthy cells, a therapy is possible by solely targeting the cancer cells. The cancer cells have a defect in a repair machinery, causing an extra injury compared to the healthy cells. Targeted therapy by means of impairing DNA repair mechanisms can be used as a single medication, but it can also be used in combination with chemo- or radiotherapy. Combining a DNA damaging agent and a DNA damage repair inhibitor, increases the cytotoxic effect and, thereby, the chance for cure.

In this literature thesis I will focus on deficiency in genes involved in the homologous recombination pathways because mutations in HR machinery, like BRCA1 and BRCA2, are very common. On the contrary, diseases like Xeroderma pigmentosum, Trichotiodystrophy and Cockayne syndrome are quite rare.

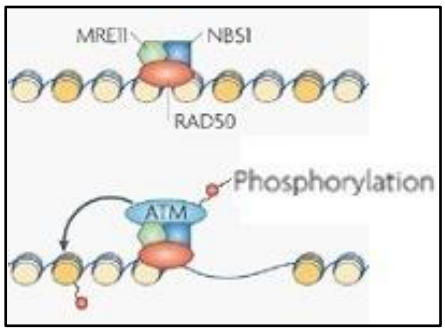
For further digging into possible mutations in the HR pathway that can cause cancer development, a closer look on the contribution of genes and their proteins is necessary.

HR repair defects as potential targets for cancer therapy

Deficiencies in DNA damage repair pathways in cancer cells are suggested to be a prospective target for cancer therapy, because healthy cells can be spared. In this literature thesis, the focus is on homologous repair, since in dividing cells, like tumor cells, DNA repair is primarily in hands, and therefore dependent of HR. A well known tumor mutation which induces impairment of HR is in the BRCA1 or BRCA2 genes. For therapeutic inhibition of a second repair pathway, PARP inhibition is proposed. By inhibition of a repair pathway for SSBs, the single strand breaks converts into DSBs during replication. In healthy cells the DSBs can be repaired, while tumor cells tend to accumulate the damage because of the lack of a proper HR, finally leading to cell death. Tumor cells with defects in NHEJ are thought to be less susceptible to inhibition of a second repair pathway, because their main and accurate DSB repair route, HR, still can restore the damages and help the tumor cells to survive. Impairment in single strand damage repair mechanisms also seems less interesting, because the SSBs are converted into DSBs during replication and can subsequently be repaired by DSB repair pathways. Therefore, the focus in this chapter is on erroneousness homologous recombination repair in different types of cancer caused by mutations in HR proteins.

Errors in HR can originate from mutations in any HR involved gene, on condition of HR dependency to that encoded protein. Genes contributing to the homologous recombination pathway, can be categorized in different stages. First the damage in the DNA has to be recognized. After this, the damage sites has to be prepared for repair and next a comparable strand needs to be found and exchanged. Once established, DNA has to be synthesized to fill up the gap arisen from the DSB and the nucleolytic processing during preparation for repair. At the end, the broken strands have to be resolved and ligated.

Recognition

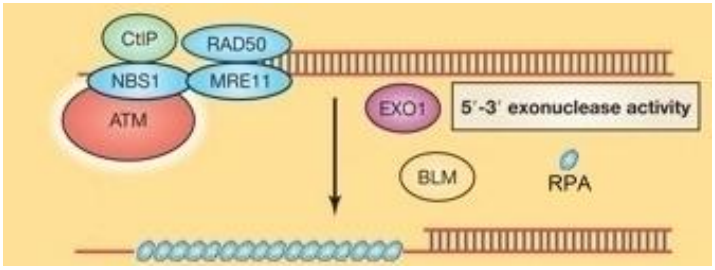


- MRN complex binds and unwinds DNA ends;
- Nbs1 and ATM interact, activating ATM phosphorylation kinase;
- ATM phosphorylates γ-H2AX as a marker for DSB
- p53 is activated to induce cell cycle arrest to facilitate DNA repair;

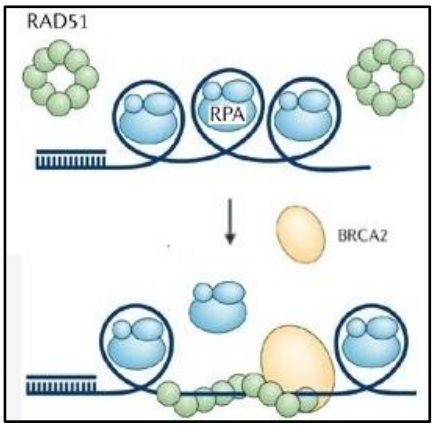
Exploration of the proteins involved which may have a crucial role in HR, leads to the first recognition protein complex MRN. ATM activation by MRN complex interaction is essential in the early HR activation. Aberrations of the **MRN complex** are shown in all three subproteins (MRE11-RAD50-NBS1). The occurrence of alterations in the NBS1 gene is pointed out earlier and is known to be responsible for the Nijmegen Breakage Syndrome. However, mutations in RAD50 (Nijmegen breakage syndrome-like disorder)[26] and the MRE11 genes (Ataxia telangiectasia-like disorder)[27,28]are also correlated with cancer. [29] Individual MRN proteins can be found heterozygous mutated (germline) in healthy cells, while silenced, homozygous mutated, in the tumors. This is consistent with the idea of loss of the heterozygosity that is suggested to induce cancer. Alterations in MRN proteins display a reduction in HR repair,[30,31,32] which leads to the expectation that MRN alterations augment the risk of cancer. Studies reveal that a decrease in, or loss of MRN complex proteins is proved in familial breast carcinomas, even more in BRCA1 or BRCA2 mutation carriers, and sporadic breast tumors. Respectively 3%, 7% and 10% tumors showed abnormally reduced protein expression for RAD50, MRE11 and NBS1, among approximately 1000 mammary carcinomas.

Second, mutations in de **ATM gene** seems to be interestingly, because of the pivotal phosphorylation activity to activate downstream substrates. In line with the predictable crucial role, ATM defects is revealed to impair HR repair.[33] The suggested cancer predisposition is confirmed as the risk of cancer increases 100-fould in individuals with ataxia-telangiectasia compared to the general population. Female patients with ataxia-telangiectasia heterozygous for an ATM mutation tend to have a 2- to 5-fold increase in risk of breast cancer.[34,35] Cancers found are lymphoid and epithelial cancers.

Processing



- MRN complex cooperates with CtIP, Exo1 and BLM resects DNA ends;
- CtIP interaction with the MRE11 complex, promotes ATR activation and recruitment of RPA.
- RPA coats ssDNA, RPA phosphorylation regulates the assembly of DNA damage induced RPA and MRN foci at both sides of the break on ssDNA.[36] RPA binding the DNA, facilitates assembly of RAD51 (and Dmc1); RAD51 has the ability to exchange the single strand with the same sequence to from a double stranded DNA molecule and forms two Holliday junctions.
- RAD51 also recruits and interacts with RAD52. Thereby, RAD52,accelerates the removal of RPA from ssDNA. RAD51 disrupts Rad52-RPA interaction and thus, RAD52 is removed from RPA[37]
- BRCA1 is recruited, inhibiting the nuclease activity of the MRN complex.
- In addition, CtIP interacts with BARD1 to inhibit the ligase activity of BRCA1/BARD1.[38]
- Complexes of BRCA1-BARD1 and BRCA2-DSS1 displaces RPA for ssDNA multimers binding of RAD51. PALB2 (FANCN) interacts en colocalizes BRCA2; BRCA1 forms a ring ligase with BARD1. The stability of BRCA2 is dependent of the presence of DSS1.[39]



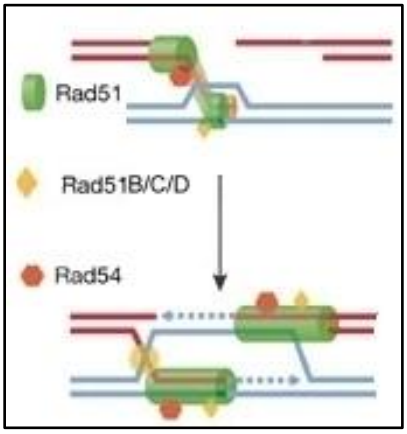
Mutations in **Replication Protein A (RPA)** seems to be likely to impair HR repair. RPA is composed of three subunits (RPA70, RPA32 and RPA14), all necessary for facilitating the binding of RAD51 and RAD52 to the DNA. The formation of RAD51 foci is decreased when RPA is reduced, leading to persistent unrepaired DSBs. A mutation in the subunit RPA70 (rfa1-t11) is known to provoke reduced removal of the RPA from the ssDNA by RAD51, thereby, making strand exchange mediated by RAD51 less efficient and impair HR repair.[40] This suggests that RPA displacement by Rad51 protein is a critical step in homologous recombination and, consequently, indicating that other mutations might lead to even greater inhibition of HR. This rises the consideration that mutations in RPA can be cancer inducing. Reported is that mice with a heterozygous mutation in RPA1 showed development of lymphomas, while homozygous mutations results in early embryonic lethality.[41] The RPA70 gene is localized at chromosome 17p13.3[42] and loss of this region has been concerned in a range of human malignancies including colorectal cancers, breast cancers, lymphomas and leukemias. However, evidence of cancers in human caused by RPA is absence. Deletions in 17p13.3 are also demonstrated in metastatic phenotype of human cancers, indicating a role of these mutations in supporting the transition from early to metastatic tumor progression.[43,44]

CtIP is used to activate resection, however, when NHEJ is favored, CtIP inhibits resection. CtIP is required for efficient homologous recombination[45] and studies demonstrated that CtIP aberrations

are present in various cancer cell lines. Confirmed is that CtIP alterations are present in various cancer cell lines,[46] thereby, mouse models shows that reduced CtIP levels lead to tumor formation.[47] This indicates that a loss of function from the CtIP can lead to a predisposition in cancer development.

It is revealed that mutations at sites crucial for the interaction between **RAD51** and BRC domains of BRCA2, impairs the ability of BRCA2 to recruit RAD51 to DSBs. Moreover, when RAD51 is absent or defect, strand invasion is impaired, obstructing recombination repair. This suggests that mutations in the RAD51 gene can contribute to a defective homologous repair and therefore, might be a risk for the introduction of cancer.[48,49]

Homology search & strand invasion:



- RAD51 searches for homology and facilitates ssDNA strands exchange (transfer to donor). Strand invasion results in the formation of a D-loop;
- DNA polymerase η can interact with RAD51, stimulating the D loop extension.[50]
- RAD54 promotes branch migration of Holliday junctions, by also capturing the second end of the break, double Holliday Junctions are formed.

Tumor genesis on the stage of the search for a homologous strand and strand invasion, are concerned with mutations in the **BRCA1 and BRCA2** pathway. As mentioned before, mutations in the BReast CAncer genes BRCA1 en BRCA2 increases the risk of developing breast and ovarian cancer. The risk of developing breast cancer in women with such mutations increases from about a 12% risk to about a 60% risk over a 90-year life span in the United States according to the National Cancer Institute. In the case of ovarian cancer, current estimations predict a 55% lifetime risk for women with BRCA1 and about 25% for women with BRCA2 mutations.[51,52] In contrast, about 1,4% of women without an inherited BRCA abnormality get ovarian cancer. The risk for certain other cancers may also be higher with BRCA1 or BRCA2 mutations.

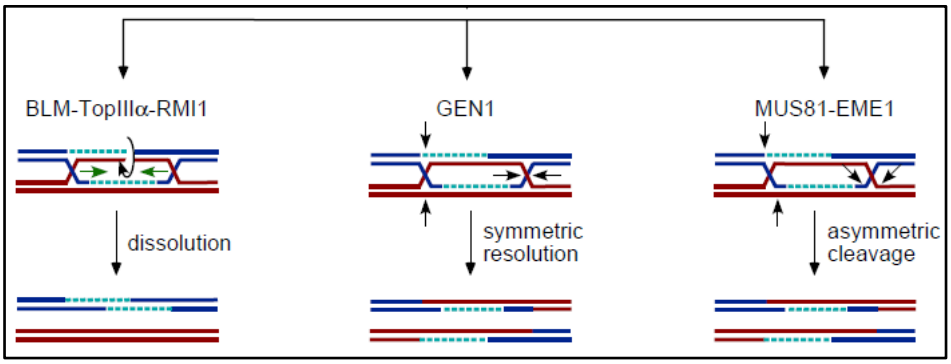
ATM and CHK2 phosphorylate BRCA1 after ionizing radiation, inducing migration of BRCA1 to γ-H2AX phosphorylated sites. BRCA1 binds directly to DNA, which contributes to its ability to inhibit the nuclease activity of the MRN complex. BRCA1 interacts indirectly with RAD51. The BRCA2 protein facilitates localization of RAD51 to sites of DNA damage and stimulates RAD51-mediated strand exchange. [53,54,55] Cells with dysfunctional BRCA1 or BRCA2, have a defective HR and, therefore, do not repair or repair their DSBs by other pathways, such as the error-prone non-homologous end joining, which probably underlies the cancer predisposition.

The BRCA pathway involves many proteins both up- and downstream, evidence is accumulating that dysfunction in these genes represent a great part of **BRCAness** mutations in sporadic cancers.[56] Mutations in one of the genes involved in the BRCA pathway, may have the prospective to impair the BRCA pathway and thereby have the ability for tumor genesis. In line with the mentioned direct interaction of DSS1 with BRCA2, deficiency of **DSS1** gives a comparable phenotype to that of BRCA2 defects. This implicates the cancer susceptibility of the DSS1 gene. Thereby, a knockdown of DSS1 in human cell lines demonstrated a striking loss of BRCA2 protein, mainly due to its increased degradation. Underlining the importance of the interaction of DSS1 and BRCA2.[57] Considering the interaction of **CTIP** (RBBP8) with BRCA1, it is likely that mutations in the CTIP gene might substitute for impairment of the BRCA1 pathway and therefore, predisposes to breast cancer. However, although CtIP seems critical for HR repair,[58] epidemiologic cancer research found no CtIP variants.[59,60]

Other proteins that cause BRCAness are those involved in Fanconi anemia pathway. [61] Alterations of **PALB (FANCN)** causes Fanconi anemia, as mentioned it localizes and provides stability to BRCA2. PALB2 mutations are identified in familial breast cancer and shows, like BRIP1, a modest elevated breast cancer susceptibility risk.[62] When **BRIP1 (FANCJ)** is heterozygous mutated, an elevated risk of breast cancer is reported, homozygous mutations cause Fanconi anemia.

Resolution:

- BLM helicase–topoisomerase IIIα–RMI1/2 dissolves the double Holliday junctions[12], or
- MUS81–EME1 cleaves asymmetrically
- GEN1 promotes Holliday junction resolution by a symmetrical cleavage



Finally, when it comes to resolution of the broken ends, mutated RecQ helicases can induce cancer. Bloom's syndrome, Werner's syndrome and Rothmund-Thomson syndrome are respectively caused by defects in the genens BLM, WRN and RecQ4 and cause premature aging. However significant effect of mutations in one of these genes leading to cancer is absent, variants have been shown to contribute to the susceptibility various cancers.[63,64,65,66,67]

PARP1 inhibition

Recently, researches focus on the BRCA repair defect as a therapeutic strategy: in addition to the HR inhibition caused by mutations, a second repair mechanism is inhibited by a Poly(ADP-Ribose)Polymerase 1 (PARP1) inhibiter. [68] PARP1 is suggested to be involved in single strand break processing and hence, inhibition of PARP1 would cause an accumulation of SSBs. However, also reports exist that describe a role for PARP in DSB repair.[69,70] This would increase the injuring effects of PARP1 inhibition, making it less suitable as targeted therapy. However, in a phase 1 clinical trial minor side-effects were monitored,[2] indicating a trivial role of PARP1 in DSB processing. This might be explained through the suggestion that PARP-1 controls DNA damage recognized by HR and that it is not involved in actual functioning of HR.[71] Another link for PARP1 with DSB repair is suggested by cooperation with DNA ligase III in an alternative type of NHEJ, that functions as backup to the classical pathway of NHEJ.[72] Because the minor involvement of PARP1 in DSB repair, the DSB repair in healthy cells should not become impaired. In addition to the involvement in DNA repair, PARP1 has also been shown to regulate gene transcription, mediate p53-regulated apoptosis and initiate cell

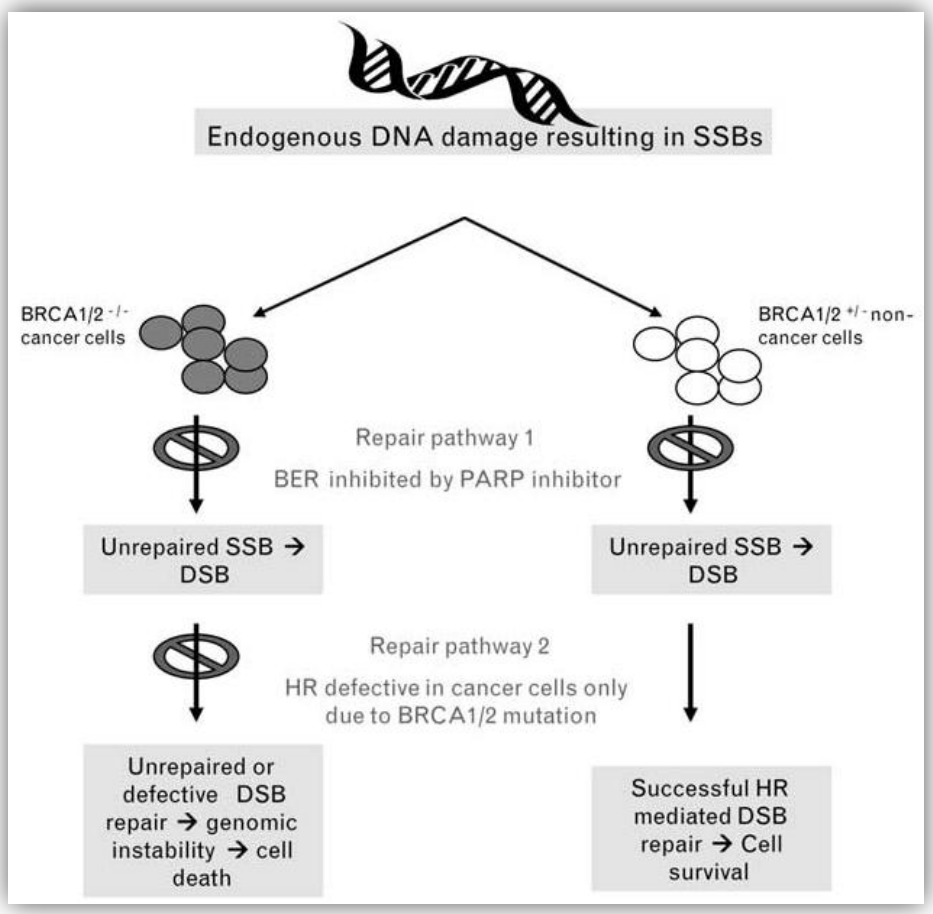


Figure 7. By inhibition of PARP1 in BRCA1 or BRCA2 deficient cells, the SSB repair is impaired, alongside the deficient HR pathways in tumor cells. Healthy cells with one, germ-line, mutation in the BRCA1 or BRCA2 allele have the ability for repair by HR. Vasileva A, Jessberger R. Precise hit: adeno-associated virus in gene targeting. Nat Rev Microbiol. 2005 Nov;3(11):837-47.

death in response to extensive DNA damage. By inhibition of PARP1, these pathways will be impaired. It is thought that the main effect of the PARP1 inhibitor is by the inhibition of the base

excision repair, so that single strand breaks will not be repaired and after replication will result in double strand breaks.

Since PARP is not involved in the HR pathway, loss of PARP activity causes a increase in damage that has to be repaired by HR. PARP inhibition in combination with a HR-impairing mutation in tumor cells, like a BRCA1 or BRCA2 mutation, therefore, causes additional silencing of the homologous recombination repair, and possibly a part of the alternative pathway in NHEJ. Consequently, double strand breaks remain unrepaired or are repaired by the error-prone NHEJ. The cancer cells will accumulate DNA damage, which leads to cell death, while healthy cells are only inhibited in single strand break repair and are able to repair DNA damage to a greater extent. A phase 1 study of Olaparib (PARP1 en PARP2 inhibitor) reports a antitumor activity in BRCA1 or BRCA2 mutations carriers.[73] Tumors with aberrations in other HR genes mentioned above, tend to be potential targets for PARP1 inhibition when impairment of HR repair is demonstrated. Accordingly, deficiencies in proteins involved in HR like RAD51, RAD54, DSS1, RPA1, NBS1, ATR, ATM, CHK1, CHK2, FANCD2, FANCA, or FANCC are verified to respond to PARP inhibition.[74]

Alternatives of PARP inhibition

Paying attention to tumors with an HR defect, therapeutic effect is likely to be maximum (since unrepaired SSBs become DSBs after replication), when a SSB repair is inhibited and consequently DSBs accumulate in cancer cells, whilst healthy cells maintain their ability to repair DSBs. Therefore, an alternative for PARP inhibition could be an enzyme involved in BER, NER or MMR. However, DNA damage does not actually has to result in a breakage, it includes also addition of a bulky adduct, uracil incorporation, removal of base or mismatch. Those damages are recognized and removed, in BER by APE, during NER by XPG and XPF–ERCC1 nuclease, in MMR by binding of MutLα or MutLβ to MutSα and thereby recruitment of EXO1, all resulting in a SSB. This suggests that inhibition of a pathway before damage is excised might prevent formation of SSBs. By this means, most effective therapy could be expected to exist from targeting enzymes after excision, thereby giving healthy cells the opportunity to repair their damages by converting them to DSB and pass them to the DSB repair pathways.

An additional effect from PARP inhibition besides preventing BER itself, is that it prevents PARP's activity as a protector of free DNA ends. Thereby, a SSB introduced in the DNA can be enlarged by degradation of the free, unprotected ends, revealing an extra effort to injure cancer cells. In addition to PARP1-mediated BER inhibion, it is demonstrated that FEN1 endonuclease activity can be inhibited, thereby blocking long patch-BER in combination with an increase of the cytotoxic effect of DNA-alkylating agent Temozolomide.[75]

During MMR the MutSα/MutLα/ MutLβ /EXO1 complex creates eventually persistent SSBs in mismatches, suggesting this complex to be a favored target to inhibit. Mutations in the MLH1 and PMS2 genes, are shown to have the ability to impair MMR in Lynch syndrome. Therefore, it is expected that inhibition of the enzymes could have a major influence as well.

In the NER pathway, XPA-RPA complex stabilizes the duplex DNA structure by means of the inhibition of strand division activity of RPA. It recognizes damaged DNA and directs cleavage of damaged DNA during NER. By means of the severe pathology of patients with an mutation in XPA (xeroderma pigmentosum), it is expected that XPA is essential for NER repair. RPA is a main ssDNA binding protein and involved in NER, HR and NHEJ.[76] Interfering with RPA's ability to bind DNA, could inhibit all of these pathways, vanishing its targeted capacity. Therefore, inhibiting RPA might be more appropriate as a common chemotherapy. XPA on the contrary, could be a potential target.

Therapy non-HR impaired tumors

Mutations in other repair pathways that cause an increased risk of cancer development, are briefly mentioned before. By impairment of other pathways than HR caused by mutations in tumors, two benefits vanish: 1) Healthy cells having the assistance of a high fidelity pathway, that is absent in cancer cells: reparation by HR is precise and mainly results in no loss of sequences in perspective with the error-prone NHEJ; 2) SSBs transferring to DSBs: every lasting SSB, is due replication fork collapse, converted into DSBs. When therapy would exist of inhibiting DSB repair, the cytotoxic effect on healthy cells increases.

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