# Promoter hypermethylation of homologous recombination repair genes: Target for therapeutic agents.

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**Abstract:**

Cells are constantly attacked during normal metabolism by agents like oxygen and free radicals which affect the DNA and result in DNA modifications such as single- and double stranded breaks (DSBs), which can ultimately result in DNA mutations. This breakage of the DNA has several negative results like chromosomal aberrations. DSBs are the target for DNA repair mechanisms, non homologous recombination (NHEJ) and homologous recombination (HR). Conservative HR is the error free mechanism where the identical sister chromatid in the late S and G2 phase is used for restoring the same sequence that was present before the break. Several cancers have genetic mutations and/or epigenetic silenced HR genes. Epigenetic silencing is a result of promoter hypermethylation in CpG rich sequences. For instance, there are a great number of studies reporting that the HR gene BRCA1 is silenced in sporadic breast cancer. Other studies report the promoter hypermethylation of the ATM and BRCA2 gene. There are many more genes involved in the HR DNA repair, of which no data of silencing are present yet. Promoter hyper methylation of HR genes could be discovered with the DNA microarray technique and could serve as a biomarker for specific HR deficient cancers and detect cells which are susceptible to cancer. When promoter hypermethylation is present, PARP inhibitors and demethylating agents could be used to treat and prevent these cancers.

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**introduction:**

The cell cycle is important for the duplication and the transmission of the genetic material to progeny cells. Cell cycle checkpoints are present which represent the restriction points between each phase of the cell cycle. The process can be halted to coordinate that each state will occur in the right sequence and in the right way before progression into the next phase (Kerzendorfer et al, 2009). DNA is constantly attacked by agents which come from the normal cell metabolism ( oxygen and free radicals) and by environmental agents (radiation and chemicals). This challenging will result in mutations, rearrangement and DNA breakage. DNA breakage can consist of single strand breakage (SSB) and DSB which are the most detrimental. DNA breaks leads to chromosomal aberration (CA) which are disruptions in the chromosome. The occurrence of CA is low in normal somatic cells and become higher in combination with the just mentioned agents. This event may cause tumor genesis by the inactivation of tumor suppressor genes or activation of once genes (Weinberg et al., 1988). There are two processes in which a cell could response to DNA damage; the signal transduction response (cell cycle arrest and apoptosis) and that of DNA damage repair. There is a network of repair mechanism present in all organisms to protect the DNA from several deficiencies.

There are two repair mechanisms defined in eukaryotic cells with reference to DSB; the homologous recombination (HR) and the non-homologous and-joining (NHEJ) (Kuschel et al, 2002). The HR mechanism is the last resort for DNA repair and when HR genes become mutated or silenced this is characterized by gross gene rearrangements (Patel et al, 1998). Anti cancer therapies are used to induce DNA damage to kill the cancer cells. Radiotherapy is the second most common therapy after surgery. Cell death is caused by ionizing radiation and associated with DSB. There are several other anti cancer drugs that also induces DSBs (Topoisomerase 2 inhibitors and cross linkers such as Melphalan) and it is known that the ability of cancer cells to repair such DSBs determines the outcome of the treatment (Helleday et al., 2010). Recent studies have reported that promoter hypermethylation of HR genes is present in breast and ovarian tumors and thus become silenced (Esteller et al., 2001). There are several genes involved in the HR and have all different functions. This functions vary from recognition of DSBs and dsDNA exonuclease activity to strand transfer. When HR genes become silenced or mutated it is known that these tumor cells are more sensitive to an anticancer drug. In this review I will shed light on the major genes involved in HR, the impact of HR on cancer with a particular focus on how HR can be used and which of this genes have hypermethylated promoters in cancer. When the promoter hypermethylation status of the HR is known, there could be other screening methods and future anticancer therapies useful instead of chemo and radiation.

**Homologous recombination:**

The long and fragile DNA is constantly damaged by several agents which result in DSBs. There are two mechanism which are capable of repairing this DSBs; homology-dependent (fig1) and –independent. The homology independent mechanism (NHEJ) is able to rejoin DSB ends. However, the NHEJ is not error free, it often creates sequence alteration at the break site. In eukaryotes both HR and NHEJ are important while in yeast only the HR is primarily used (Gent et al, 2001). HR requires long sequence homologies of several hundred base pairs to restore the original sequence at the DSB site, this mechanism of DSB repair is error-free. HR is subdivided in conservative HR and non-conservative. Here, I will focus on the conservative HR mechanism. HR is found in meiosis where it has a function to create genetic diversity (Keeney et al., 2001) and in mitotic cells it has a function in the DSB repair. Conservative HR is also called gene conversion because the repair is achieved by copying the sequence information of the sister chromatid, resulting in two intact copies. After an DSB is induced, the 5'→3' will be exonucleolytically resected to acquire long 3' single-stranded tails. This long single stranded tail invades the intact DNA duplex at the site of sequence homology. This invasion into the homologous strand result in the formation of a displacement-loop (D-loop). In this D-loop formation the 3' ends of the invading loop serve as primers for repair synthesis. DNA polymerase synthesize the new strands so that on new strand present in the donor and the recipient. This pairing is followed by formation of a Holliday junction intermediate, migration and resolution (Holliday et al., 1964). Endonucleolytic resolution resolve the junction to restore two linear DNA duplexes. HR in meiotic dividing cells results in gene conversion with cross-over. This crossing-over is the result of HR between homologues. In mitotic cells HR is in most of the time without crossing over (factor 100-1000). HR in mitotic cells occur between identical sister chromatids so that the sequence that was present before the breakage is restored at the break side (Johnson et al., 2001). Identical sister chromatids are only present in late S phase and G2 phase when the chromosome is duplicated, for this reason is HR active in late S phase and G2 phase mitotic cells. (fig2)

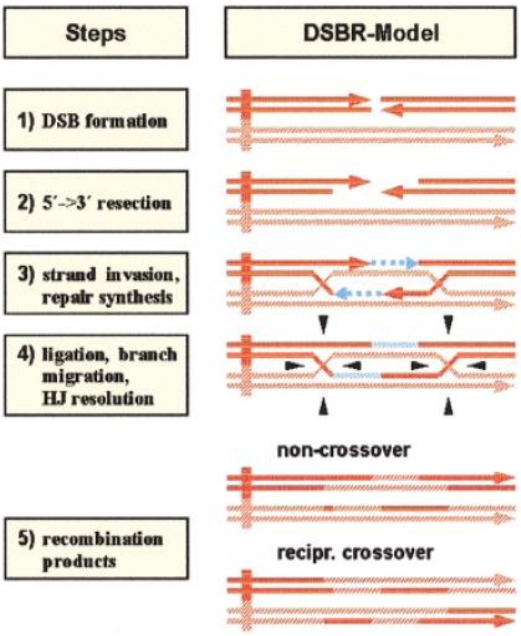


fig 1. Steps involved in DSB repair. After the DSB is induced, long 3' ssDNA is formed. Strand invasion result in D-loop formation where the long 3' ssDNA serve as a primer for repair synthesis. Repair is followed by holiday junction intermediate, migration and resolution followed by the resolving the junction to obtain two linear DNA duplexes. (Pfeiffer et al., 2000)

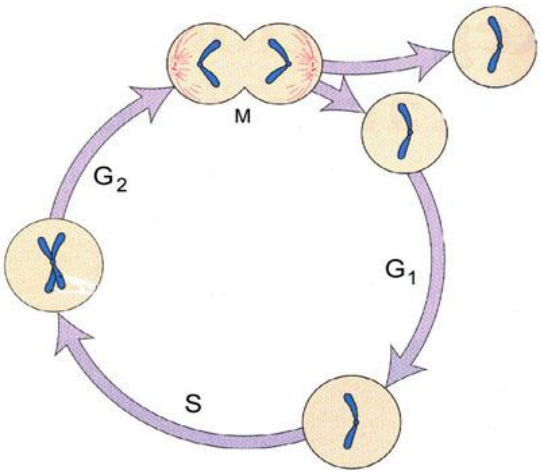


Fig 2. identical sister chromatids present in late S and G2 phase. (picture obtained from the medical biochemistry page)

**Genes involved in HR and their functions:**

Several genes have been identified since the rapid development of specific recombination assays. It is important to note that HR and NHEJ recombination compete with each other but some genes involved in HR are also involved in the NHEJ pathway. There are many genes involved in the HR. Here, I will discuss the major genes involved in the HR pathway. (fig 3)

ATM:

The ataxia telangietasie mutated gene (ATM) is a kinase and is recruited and activated after DNA damage. Both ATM and the related ATR protein have a function in cell cycle arrest and DNA repair (Hoekstra et al., 1997). This enzyme phosphorylate several substrates in cell cycle regulation and DNA repair like P53, BRCA1 and other tumor suppressor genes. Loss of ATM increases genomic instability and is associated with the autosomal recessive disorder ataxia telangietasie. ATM is recruited to the DSB via a complex composed of three other HR genes: MRE11-RAD50-NSB1 (MRN) (Lee et al., 2004).

MRN:

The MRN complex recognizes DSB. The MRN complex may have a major function in the creation of ssDNA at sites of DSB. The Mre11 and the RAD50 proteins bind to the broken ends of the DNA and attach them to each other. The NBS protein activates the ATM kinase and this converts the inactive ATM dimer to active ATM monomers. This activated ATM kinase phosphorylates several substrates involved in the repair mechanism (Czornak et al., 2008)

Mre11:

The Mre11 protein dimerizes to form an U-form dimer, this U-form is needed for the MRN function, required for DNA binding and repair activity (Williams et al., 2008). In vitro

studies have demonstrated that Mre11 has dsDNA exonuclease activity (Paul et al., 1999). This exonuclease activity is required to make long 3' single stranded tails which serve as primers for repair synthesis

RAD50:

The RAD50 protein has a function in the sister chromatid cohesion, DNA binding, partial unwinding and ATPase activity (Chen et al., 2005). The N and C terminus of the protein specific motifs required for the ATPase activity. The middle region of the protein contain of a large coiled-coil region which can fold itself back via a hinge region in the central. The hinge region consists of zinc motif which allows RAD50 to become an dimer (Hopnfer et al., 2003). The Mre11 protein binds to RAD50 near its A en B motifs.

NBS1:

The enzymatic function of the NBS1 protein is not known yet, although it is known that it regulates the MRN and BRCA function. NBS1 is needed for the localization of RAD50 en Mre11 (Shima et al., 2005), the rapid assembly of the MRN complex to the DSB and it stimulate the activity of RAD50 and Mre11 by activation of the ATM protein.

RAD51:

The main function of the RAD51 protein is the strand transfer between the broken DNA strand and its homologue to allow repair of the broken sequence (West et al., 2003). In vertebrates the absence of the RAD51 protein is lethal. Strand invasion implies, polymerizing of RAD51 on the 3' of the DNA end and intervening in the transfer and the annealing of the protein filament tot the homologue on the sister chromatid. This strand invasion results in the earlier mentioned D-loop which make DNA synthesis possible. Recent studies showed that there are several RAD51 like proteins with similar functions. This proteins were called, XRCC2, XRCC3, RAD51L1, RAD51L2 and RAD51L3 and have high similarity in their ATP-binding domain (Thacker et al., 1999). RAD51 also interacts with RAD54 and RAD52, in both cases the function is unknown.

BRCA1:

The BRCA1 protein is thought to have a early roll in the promotion of HR. ATM or ATR phosphorylates BRCA1 in response to DNA DSB (Cortez et al., 1999). BRCA1 is a component of a large complex of several proteins like BASC which can influence the choice of repair pathway. BRCA1 is known to play a specific role in the regulation of DSB processing by the MRN complex. The BRCA1 protein binds DNA directly and when it does bind it inhibit the function of Mre11 (Paul et al., 2001). I suggest that after Mre11 has exonucleolytically made long 3' ssDNA, the BRCA1 protein binds the DNA to prevent that too long ssDNA is formed.

BRCA2:

The BRCA2 protein is more important than the BRCA1 protein and plays a more central role in the HR DNA repair through interaction with RAD51. BRCA2 binds to the RAD51 protein via eight conserving binding domains, the BRC repeats (Pellegrini et al., 2002). The C terminal domain of BRCA2 is capable of binding to ssDNA which is critical for HR promoting. BRCA1 and BRCA2 both contain nuclear localization signals (NLS). This NLS is not present in all DNA repair proteins, therefore are the BRCA proteins the transporter for other proteins into the nucleus where they have repair activities of DSBs (Davies et al., 2001).

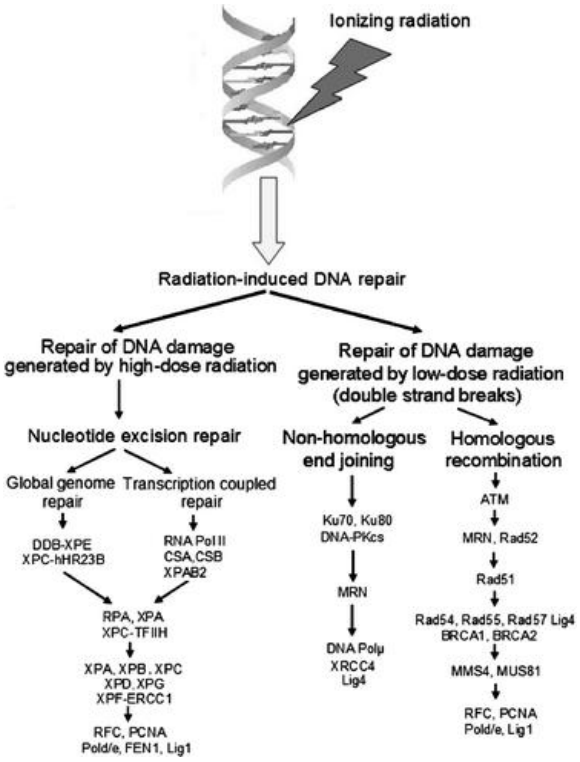


fig 3. Genes involved in DNA damage repair after radiation.

**Promoter hypermethylation:**

Different patterns of methylation and demethylation regulate the cellular growth and differentiation in the early embryogenesis. DNA methylation occurs mainly at the cytosine residue of a CpG dinucleotide where a methyl group is bound to the 5-carbon position of the cytosine pyrimidine ring (Zhang et al., 2005). This CpGs are scattered unequally within the genome and have a high prevalence in CpG islands. CpG islands are regions within the genome that contain a high frequency of CpG sites. The definition of a CpG island is a region with at least 200 base pairs and a CG percentage of 50% or more, and a CpG region greater than 60%. The p refers to the phosphodiester between the C and the G (Bird, 2002). The methylation pattern is transmitted to daughter cells during replication catalyzed by DNA methyltranferase, conserving the methylation pattern (Herman et al., 2003). In mammalian genomes, CpG islands are 300-3000 base pairs in length. They are found in and near 40% of promoters of genes, especially in housekeeping genes (Razin, 1998). These CpG islands normally occur at or near the transcription start site of genes. DNA methylation of a CpG island in a promoter gene may inhibit the expression of a gene and prevents transcription factors to access DNA, while downstream methylation has no influence on the expression (Down et al., 2002). CpG methylation at promoter regions, resulting in gene silencing, is a fundamental event in carciogenesis, the most common cause of tumor suppressor gene inactivation and therefore called epigenetic mutations (Bonazzi et al.,2009). There are several genes that cause specific cancer when they are inactivated by hypermethylation. Examples include RB1, VHL, MLH1, BRCA1 and APC.

**Promoter hypermethylation in HR genes:**

Oxygen and free radicals are produced during the normal metabolism which result in DNA damage that need to be repaired to maintain genome stability. Previous studies have shown that oncogenes produce damage at the replication forks; replication stress (Di Micco et al., 2006), which emphasize that tumors also need to activate DNA damage repair. As mentioned earlier, HR is the major pathway to repair this DNA damage. There are several HR proteins associated with cancer, examples include, BRCA1 and BRCA2 in ovarian and breast cancer (Miki et al., 1994) (Wooster et al., 1995), Rad51 in uterine leiomyoma and lipoma (Schoenmakers et al., 1999) and Nbs1 in lymphoid cancer (Matsuura et al., 1998). The genes of this proteins could be mutated, or silenced as a result of promoter hyper methylation. The just mentioned HR proteins involved in cancer are all mutated and therefore have a dominant negative effect.

BRCA1 or BRCA2 germline mutations are known to increase the risk of developing breast and ovarian cancer. The loss of wild-type allele result in genome instability due to the inactivation of HR. In some sporadic breast cancers are somatic mutations not found while there is reduced gene expression of the BRCA1 protein (Futreal et al., 1994). As noted earlier, this is the cause of promoter hypermethylation of the BRCA1 gene. Promoter hypermethylation was not the case in the BRCA2 gene in sporadic breast cancer (Birggisdottir et al., 2006). The ATM gene plays a major role in the DNA repair pathway by the phosphorylation of several substrates which are involved in the HR and the NHEJ. Previous studies have shown that promoter hypermethylation of the ATM gene is associated with head and neck squamous cell carcinoma (HNSCC). In a study was shown that promoter hypermethylation was present in 25% of HNSCC (AI L. et al. 2004). The NBS1 gene is involved in the MRN complex and mutations are associated with acute myeloid leukemia (AML) (Resnick et al., 2002). There is a strong association of NBS with childhood hematological malignancies and could also be involved in sporadic childhood leukemia. A recent study have shown that no promoter hypermethylation was present in any CpG island of the NSB1 gene in leukemia samples (Meyer et al., 2006). The promoter methylation status of the genes Mre11, RAD50 and Rad51 is not described in the present literature. In table 1 I present the promoter hypermethylation status of the major genes involved in HR, these date of the HR genes are obtained from cancer cells.

table 1. Promoter hypermethylation status of the major Genes involved in HR.

| Gene | Methylation status | Source |
|---|---|---|
| ATM | **+** | AI L. et al., 2004 |
| Mre11 | x | x |
| Rad50 | x | x |
| NBS1 | **-** | Meyer S. et al., 2006 |
| Rad51 | x | x |
| BRCA1 | **+** | Honrado E. et al., 2007, Birggisdottir et al., 2006 |
| BRCA2 | **+/-** | Cucer N. et al., 2008, Birggisdottir et al., 2006 |

**Cancer and the cell cycle**

In tumor cells are several pathways disturbed or inactive that control the proliferation/cell cycle response. There are many pathways in which the cell cycle becomes deregulated. One pathway that is mutated and responsible for tumor development is the disruption of the mitogenic external signals (Hunter, 2000). These mutation in this pathway may include autocrine production of mitogen, mutations that activate the mitogen receptor tyrosine kinases (RTKs), G-proteins such as RAS and mutations in one of signal transducing molecules responsible for the transport of mitogenic information (Blume-Jensen et al., 2001). Another pathway includes those that target the late G1 phase of the cell cycle which

is regulated by the RB protein (Harbour et al., 2000). Mutations in the pathway include deletions in the RB gene and deregulation of the CDKs which regulate the activity of the RB protein (Sherr, 1996). A third pathway in which the cell cycle become deregulated is the uncontrolled expression of the Myc protein. The Myc protein is tightly controlled by the presence of mitogen but is overexpressed in tumor cells (Ellend et al., 1999).

**Treatment:**

The common way to treat cancer is to expose the body to substances that kill tumor cells and do not damage the normal body cells. When trying not to damage the normal body cells, specific cellular features have to be discovered which only eliminate the cancer. It is of generally knowledge that cancer cells proliferate more rapidly than normal body cells as a result of the just mentioned disturbed or inactive pathways that control the proliferation/cell cycle response. Therefore are several cancer therapies targeted on the cell cycle. Cell division can be manipulated by blocking the mitotic spindle which result in unequal division of the two daughter cells. Growth signals can be manipulated by hormones, antibodies and inhibiting drugs. The most common used therapy is drugs which induce DNA damage. DNA damage drugs result in cell cycle arrest and cell death. Replication associated DSBs occur when lesions are present in the S phase by blocking the replication fork. Cells trying to replicate this damaged DNA may result in increased cell killing, so DNA damaged cells has a higher mortality rate compared to normal cells which divide less frequent. The survival rate of cells with DNA damage is determined by the ability to repair the damaged DNA. The effectiveness of therapies which induce DNA damage can be altered by the repair pathways. Therefore is DNA repair a promising target for novel cancer treatments. When DSBs occur directly they are repaired by NHEJ (Sargent et al., 1997) and replication associated DSBs are repaired by the HR and other related pathways (Arnaudeau et al., 2001). Small lesions and DNA adducts may be removed and repaired before the DNA become replicated. Base-excision repair (BER) removes a damaged short strand or a single damaged DNA base (Sharma et al., 2007) and nucleotide excision repair (NER) removes a short ssDNA containing the DNA lesion or adduct (Sugasawa et al., 2001). The replication fork stalls or collapses when this lesions and adducts are not removed and other repair pathways have to become activated. Collapsed replication forks will result in DSBs, cell cycle arrest, DNA repair, apoptosis or senescence (Kastan et al., 2004).

Surgery, chemotherapy and radiotherapy are used to treat patients with cancer. The most commonly used platinum chemotherapy in the treatment of cancer are cisplatin, carboplatin and oxaliplatin. These platinums crosslinks DNA in cells which are present just before cell division, which result in DNA damage. Platinum resistance is a major problem and this is accomplished by the DNA repair pathways which repair the lesions in the DNA produced by the chemotherapy. Inhibitors of the DNA repair are used to make tumor cells more sensitive to chemotherapy.

**HR in treatment:**

The ultimate goal of chemotherapy is to damage DNA in a way that it will result in cell cycle arrest and cell death. Chemotherapy is not the only cause of DNA damage, as noted earlier it also occurs spontaneously and the DNA repair of normal cells is required. as noted earlier are some DNA repair

mechanisms mutated or silenced and thus inactivated. When both alleles of a DNA repair gene are mutated or silenced in a tumor cell, DNA repair inhibitors could be used as a monotherapy. HR DNA repair is the most used mechanism to repair replication lesions which are formed by anticancer drug. HR takes place in the late S phase and the G2 phase because this pathway needs identical sister chromatids (fig3). Cancer cells have the characteristic of uncontrolled growth, so a high percentage of tumor cells resides in the S phase and G2 phase. Increased knowledge about the creation of lesions and the mechanism that repair this lesions has been discovered the last decade. With this perceived knowledge, tumor cells killing has been increased due to the combination of cytotoxic drugs and inhibitors of the DNA repair pathway. Now-a-days there are several inhibitors of the DNA repair pathway developed and are used in clinical trials to see what their effect is (Helleday et al., 2008). Poly (ADP-ribose) polymerase 1 (PARP1) inhibitor is one of the drugs that inhibits a DNA repair pathway. The PARP1 protein is required for the BER of DNA lesions (Schreiber et al., 2006). The PARP1 protein consists of six domains and binds with the two zinc finger domains to the single stranded breakage (SSB). When the protein binds the SSB it catalyzes several substances and attract enzymes which are necessary for the BER. PARP1 inhibitors were first use in combination with chemotherapy to block the reparation of the chemo induced lesions. DNA repair inhibition as a monotherapy have been demonstrated to work in embryonic stem (ES) cells lacking wild type BRCA1 and BRCA2 (Farmer et al., 2005). Another study used primary inherited breast and ovarian cancer cells which lack the wild type of the BRCA2 gene (Bryant et al., 2005), both studies showed increased apoptosis and cell cycle arrest of the tumor cells. In both studies were PARP inhibitors used as a monotherapy.

BRCA1 and BRCA2 mutated or silenced proteins result in replication associated lesions and cannot repair DSB by homologous recombination (Lomonosov et al., 2003). These HR inactive cells are 100-1000 fold more sensitive for PARP inhibitors than normal wild type cells (Bryant et al., 2005). The use of monotherapy PARP inhibitors makes no difference between mutated or silenced HR genes. In both situations is the protein inactive and therefore no longer capable of repairing DSB by HR. The function of PARP inhibitors is to block the repair of a single damaged DNA base/ short single stranded damage which will result in DSBs. PARP1 inhibition itself is not toxic. Normal cells will repair this damage by the HR mechanism which operate error free. HR defective cells are not capable of repair this damage error free and have to use the NHEJ which will result in CA. For this reason the PARP inhibition are selectively lethal for cancer cells. (Fig4.) 10 to 15% of woman who develop ovarian or breast cancer carry the BRCA1 or BRCA2 germline mutation, but dysfunction of the BRCA1 and BRCA2 is much more prevalent. This dysfunction of the BRCA1 and BRCA2 is called BRCAness (Turner et al., 2004). One way to develop BRCAness is the promoter hypermethylation of the BRCA genes. There are several HR genes in which the promoter hypermethylation can create the BRCAness phenotype. It is a challenge to develop a method to identify BRCAness and BRCAness phenotype, so that patients can benefit from PARP inhibition therapy.

When a specific gene of the HR is mutated or silenced in cancer is the PARP inhibition is effective, but there could be another way in which, specific the silenced, HR genes could be used in the treatment of cancer. As noted before are mutated and/or silenced HR genes probably the cause of genetic instability and may cause tumor genesis by the inactivation

of tumor suppressor genes or activation of once genes (Weinberg et al., 1988). I suggest that, due to selective forces driving carciogenesis, specific HR genes become methylated that promote further genetic defects which are required for the genesis of tumors. It could be no coincidence that many mutated and silenced HR genes are associated with several types of cancers. Genetic mutations automatically passed on to daughter cells through replication, epigenetic mutations are not passively inherited and have to be actively maintained by the methyl transferase enzyme. Silenced genes due to promoter hypermethylation could be reactivated by methyl transferase inhibitors and is therefore an therapeutic target (fig5.)

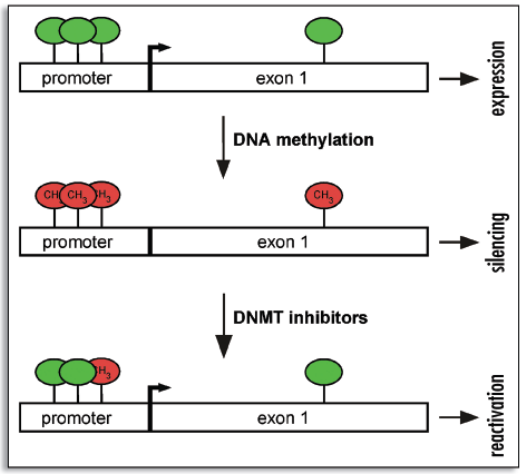


fig 5. DNMT inhibitors reactivate silenced genes. In healthy cells, HR genes are unmethylated and expressed at normal levels. In cancer cells, hypermethylation of the promoter region leads to gene silencing. Treatment with DNA methyltranferase inhibitors (DNMT inhibitors) can reactivate gene expression. (picture obtained from Landes Bioscience)

The reactivation of silenced HR genes could therefore be used in a therapy to prevent the genesis of tumors. There are several DNA methyltranferase (DNMT) inhibitors available and present in clinical trials. There are two pathways in which the DNMT inhibitors block the function of DNMT; nucleoside inhibitors and nonnucleoside inhibitors. (fig6.) The nucleoside inhibitor 5-azacityde incorporates into the DNA after it has been chemically modified and serve as suicide substrates for DNMT enzymes (Jones et al., 1980). This modification is necessary because 5-azacityde is a ribose nucleoside and have to be converted to deoxyribonucleoside. A portion of the unmodified 5-azacityde binds the RNA which affect several RNA functions (Cihak, 1974). An example of a nonnucleoside inhibitor of the DNMT is the local anesthetic procaine. Procaine is thought to bind the CpG islands which are the regions where methylation takes place. DNA methyltranferase is not capable of binding the CpG rich sequences when procaine is bound (Villar-Garea et al., 2003).

Silenced genes involved in the HR become reactivated after treatment with demethylating agents and could therefore inhibit the genesis of tumors. For this reason could the early detection of promoter hypermethylation in HR genes server as a biomarker for cancer and specific drugs can be used to reactivate this genes. Reactivation of HR genes are only useful when there are no tumors detected yet, so this could serve as a preventive treatment for normal cells where specific HR genes are silenced.

**Future perspectives:**

As previous described, not all the HR genes have been reported to show hypermethylation in tumors. This absence of information does not mean that there is no promoter hypermethylation present in this genes. The common used method to screen for candidate genes involved in cancer is to look for mutations. The epigenetic silencing through methylation is a novel approach to look for genes involved in pathogenesis. Maybe there is HR gene promoter hypermethylation present in cancer but not yet discovered. Promoter hypermethylation result in silencing of the gene and thus

absence of mRNA and protein. DNA microarrays could be used to compare HR genes in several tumor cells and normal cells. Differences in gene expression of HR genes could be measured. When there are significant differences between tumor cells and normal cells methylation specific PCR could be used to check if promoter hypermethylation is present. In this way is the screening for the pathogenesis of several cancers become elaborated and more treatment possibilities will be available. When specific HR genes are silenced in cancer, this genes could be targeted with PARP inhibitors. When silencing of HR genes is detected in normal body cells, they can be treated with demethylating agents to prevent that these cells will become tumors.

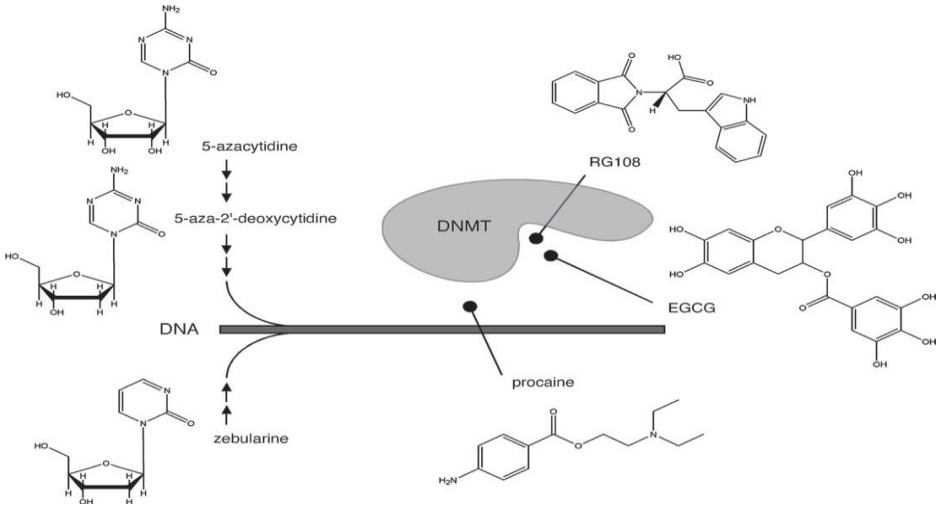


fig 6. Examples of nucleoside – and nonnucleoside inhibitors of the DNMT enzyme (lyko et al., 2005)

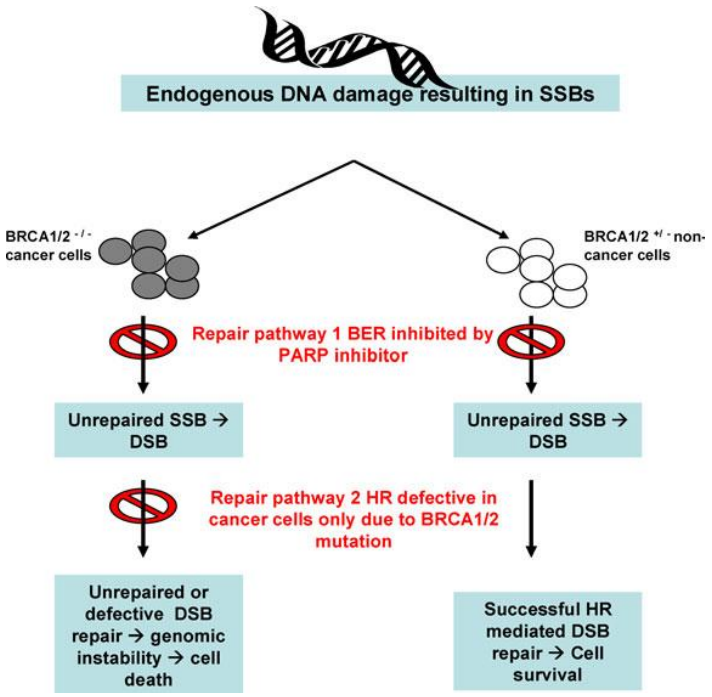


Fig 4. PARP inhibition selectively kill cancer cells (Drew et al., 2008)

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