The Role of DNA Repair in the Onset and Therapy of Ovarian Cancer

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Čestné prohlášení

Prohlašuji, že jsem bakalářskou práci na téma: “The Role of DNA Repair in the Onset and Therapy of Ovarian Cancer“ vypracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 12. 5. 2017

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Kristýna Tomášová
Abstract

DNA repair and DNA damage response are very important biological systems, inevitable to maintain genomic stability and fidelity of the genetic information, for the onset of ovarian cancer. Further, DNA repair is also substantially involved in the response to the therapy, since many chemotherapeutics act as DNA damaging agents.

This literary analysis is intended to survey the relevance of DNA repair to ovarian carcinogenesis. Special emphasis is placed on repair defects, as it is inextricably associated with the onset of cancer and treatment outcome. Apart from well-known alternations in ovarian cancer susceptibility genes, such as BRCA1 and BRCA2 involved in homologous recombination repair, ample space will be dedicated to less common gene mutations across different repair pathways.

Research confirms that abnormalities in the proteins responsible for homologous recombination repair are the leading cause of ovarian cancer. The majority of authors also suggested that targeting DNA repair pathways, especially base excision repair, can improve chemotherapy efficiency in a synergic manner. The same applies to nucleotide excision repair, which repairs platinum-DNA adducts and thus contributes to platinum drugs resistance emerging. By way of contrast, mismatch repair in ovarian cancer is rather poorly investigated and its deficiency is frequently related to Lynch syndrome, which predispose people to get colon as well as extracolonic cancers.

Key Words: DNA damage, DNA repair, ovarian cancer, incidence, therapy, resistance towards chemotherapy
Abstrakt

Detekce poškození DNA a její oprava jsou důležité biologické systémy, které chrání genetickou informaci před změnami a přispívají tak k celkové stabilitě genomu. Jelikož je mnoho látek užívaných v boji proti rakovině založených na vyvolávání DNA poškození, podílí se mechanizmus oprav DNA významnou měrou i na léčebné odezvě organismu na chemoterapii.

Cílem této práce je uceleně shrnout problematiku vzniku rakoviny vaječníků ve vztahu k opravě DNA. Zvláštní důraz je přitom kladen na poruchy jednotlivých reparačních druh, které jsou neoddělitelně spjaty se vznikem rakoviny i úspěšnosti její léčby. Kromě nejznámějších genových mutací souvisejících s rakovinou vaječníků, jako jsou BRCA1 a BRCA2 účastnící se homologní rekombinace, bude značný prostor věnován i méně známým mutacím.

Nejčastějším důvodem vzniku rakoviny vaječníků jsou dle výzkumů poruchy funkce proteinů uplatňujících se v opravách DNA homologní rekombinaci. Výzkumy rovněž ukazují, že cílená inhibice či poškození opravných druh DNA, zejména bázových excizních oprav, mohou napomáhat efektivnímu působení chemoterapie. To platí i pro nukleotidové excizní opravy, které odstraňují adukty platinových komplexů na DNA a napomáhají tak k ustanovení rezistence vůči platinovým cytostatickým. Mechanismus korekce správného párování bazí je u ovarialního karcinomu oproti výše zmínovaným drahám dosud poměrně málo prozkoumán. Porucha tohoto systému je nejčastěji zmiňována v souvislosti s Lynchovým syndromem, onemocněním projevujícím se vznikem kolorektálního karcinomu a několika dalších typů rakovin včetně rakoviny vaječníků.

Klíčová slova: poškození DNA, oprava DNA, nádory vaječníků, incidence, léčba, resistence k cytostatické léčbě
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<tr>
<td>A</td>
<td>adenine</td>
</tr>
<tr>
<td>ABC transporter</td>
<td>ATP-binding cassette transporter</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>AKT1</td>
<td>AKT serine/threonine kinase 1</td>
</tr>
<tr>
<td>AKT2</td>
<td>AKT serine/threonine kinase 1</td>
</tr>
<tr>
<td>AP</td>
<td>apurinic/apyrimidinic site</td>
</tr>
<tr>
<td>APE1</td>
<td>AP endonuclease 1</td>
</tr>
<tr>
<td>APEX</td>
<td>gene encoding AP endonuclease 1</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
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<tr>
<td>BCDX2</td>
<td>protein complex consisting of RAD51B, RAD51C, RAD51D and XRCC2 subunits</td>
</tr>
<tr>
<td>BCL-2</td>
<td>gene encoding B-cell lymphoma 2 apoptosis regulator</td>
</tr>
<tr>
<td>BER</td>
<td>base excision repair</td>
</tr>
<tr>
<td>BRAF</td>
<td>gene encoding serine/threonine protein kinase B-Raf, proto-oncogene</td>
</tr>
<tr>
<td>BRAF(V600E)</td>
<td>mutation at codon 600 of BRAF, where the valine is substituted by glutamic acid</td>
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<tr>
<td>BRCA1</td>
<td>gene encoding breast cancer type 1 susceptibility protein</td>
</tr>
<tr>
<td>BRCA2</td>
<td>gene encoding breast cancer type 2 susceptibility protein</td>
</tr>
<tr>
<td>BRIP1</td>
<td>gene encoding BRCA1 interacting protein C-terminal helicase 1</td>
</tr>
<tr>
<td>C</td>
<td>cytosine</td>
</tr>
<tr>
<td>c-myc</td>
<td>myc proto-oncogene protein</td>
</tr>
<tr>
<td>CAK</td>
<td>CDK-activating kinase complex</td>
</tr>
<tr>
<td>CAV1</td>
<td>caveolin-1, scaffolding protein</td>
</tr>
<tr>
<td>CDK12</td>
<td>gene encoding cyclin dependent kinase 12</td>
</tr>
<tr>
<td>CMMRD</td>
<td>constitutional mismatch repair deficiency</td>
</tr>
<tr>
<td>CpG island</td>
<td>cytosine-phosphate-guanine island</td>
</tr>
<tr>
<td>CRC</td>
<td>colorectal cancer</td>
</tr>
<tr>
<td>CSA</td>
<td>Cockayne syndrome A protein</td>
</tr>
<tr>
<td>CSB</td>
<td>Cockayne syndrome B protein</td>
</tr>
<tr>
<td>CX3</td>
<td>protein complex consisting of RAD51C and XRCC3 subunits</td>
</tr>
<tr>
<td>DDB1</td>
<td>DNA damage-binding protein 1, UV-induced DNA damage recognizing protein</td>
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<tr>
<td>DDB2</td>
<td>DNA damage-binding protein 2, UV-induced DNA damage recognizing protein</td>
</tr>
<tr>
<td>DDR</td>
<td>DNA damage response</td>
</tr>
<tr>
<td>DHJ</td>
<td>double holliday junction</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNA2</td>
<td>DNA replication helicase/nuclease 2</td>
</tr>
<tr>
<td>dRP</td>
<td>deoxyribophosphate</td>
</tr>
<tr>
<td>DSB</td>
<td>double strand break</td>
</tr>
<tr>
<td>DSBR</td>
<td>double strand break repair</td>
</tr>
<tr>
<td>dsDNA</td>
<td>double-stranded deoxyribonucleic acid</td>
</tr>
<tr>
<td>e.g.</td>
<td><em>exempli gratia</em>, latin phrase meaning „for example“</td>
</tr>
<tr>
<td>EC</td>
<td>endometrial cancer</td>
</tr>
<tr>
<td>EOC</td>
<td>epithelial ovarian cancer</td>
</tr>
<tr>
<td>ERCC1</td>
<td>excision repair cross-complementation group 1</td>
</tr>
<tr>
<td>et al.</td>
<td><em>et alii</em>, latin phrase meaning „and others“</td>
</tr>
<tr>
<td>etc.</td>
<td><em>et cetera</em>, latin phrase meaning „and so forth“</td>
</tr>
<tr>
<td>EXO1</td>
<td>exonuclease 1</td>
</tr>
<tr>
<td>FA</td>
<td>Fanconi anemia</td>
</tr>
<tr>
<td>FANCD2</td>
<td>Fanconi anemia complementation group D 2 protein</td>
</tr>
<tr>
<td>FEN-1</td>
<td>flap endonuclease 1</td>
</tr>
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<td>Fig.</td>
<td>figure</td>
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G  guanine
GADD45 gene encoding growth arrest and DNA-damage-inducible proteins, cell cycle regulators
GGR  global genomic NER
HBOC  hereditary breast and ovarian cancer syndrome
HELQ  HELQ helicase
HER-2/neu  human epidermal growth factor receptor 2
HJ  holliday junction
HNPCC  hereditary nonpolyposis colorectal cancer
HR  homologous recombination
HRR  homologous recombination repair
ICL  interstrand crosslink
KRAS  v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
KRAS(G12C)  mutation at codon 12 of KRAS, where the glycine is substituted by cysteine
LIG1  DNA ligase 1
LIG3  DNA ligase 3
LLS  Lynch-like syndrome
LS  Lynch syndrome
MAP  MUTYH-associated polyposis
MBD4  gene encoding methyl-CpG-binding domain protein 4, DNA glycosylase
MLH1  gene encoding mutL homolog 1, MMR component
MMC  mytomycin C
MMEJ  microhomology-mediated end joining
MMR  mismatch repair
MPG  N-methylpurine DNA glycosylase
Mre11  double strand break repair nuclease
MRN complex  Mre11-RAD50-NBS1 protein complex involved in initial DSB response
MSH2  gene encoding mutS homolog 2, MMR component
MSH6  gene encoding mutS homolog 6, MMR component
MSI  microsatellite instability
MutH  methyl-directed mismatch repair protein MutH
MutL  methyl-directed mismatch repair protein MutL
MutLa, MutLβ, MutLγ  forms of MutL protein
MutS  methyl-directed mismatch repair protein MutS, heterodimer
MutSa, MutSβ  forms of MutS protein
MUTYH  MutY DNA glycosylase
NBS1  nibrin, a protein encoded by NBN gene
NEIL1  endonuclease VII-like 1
NEIL2  endonuclease VII-like 2
NER  nucleotide excision repair
NHEJ  non-homologous end joining
OvC  ovarian cancer
OGG1  gene encoding 8-oxoguanine glycosylase
p  phosphate
p8  transcriptional regulator p8
p34  protein kinase p34
p44  protein activator p44
p52  matrix protein p52
p53  tumor suppressor protein p53 encoded by TP53 gene
p62  ubiquitin-binding protein p62
P-gp  P-glycoprotein 1 encoded by MDR1 gene
PARP  Poly-ADP ribose polymerase
PARPis  Poly-ADP ribose polymerases inhibitors
PCNA  proliferating cell nuclear antigen, DNA clamp
PFI  platinum free interval
PI3K/Akt pathway  phosphatidylinositol-4,5-bisphosphate 3-kinase / AKT serine/threonine kinase pathway
PMS2  PMS1 homolog 2, MMR component
PNKP  polynucleotide kinase 3’ phosphatase
pol β  DNA polymerase β
pol δ  DNA polymerase delta
pol ε  DNA polymerase epsilon
pol θ  DNA polymerase theta
POLQ  gene encoding DNA polymerase theta
pRb  retinoblastoma protein encoded by RB gene
PTEN  phosphate and tensin homolog
RAD23B  RAD23B Homolog B, NER protein
RAD50  RAD50 double strand break repair protein
RAD51  RAD51 recombinase
RAD51C  gene encoding Rad51 paralog C
RAD51D  gene encoding Rad51 paralog D
RAD52  DNA repair protein RAD52
RFC  replication factor C
RNA  ribonucleic acid
RNAP II  RNA polymerase II
RPA  replication protein A, heterotrimer composed of RPA1, RPA2 and RPA3
SDSA  synthesis-dependent strand annealing
SLC transporter  solute carrier transporter
SNP  single nucleotide polymorphism
SSB  single strand break
ssDNA  single-stranded deoxyribonucleic acid
T  thymine
TCR  transcription coupled NER
TFIIH  transcription factor II human
TP53  gene encoding tumor suppressor protein p53
TSG  tumor suppressor gene
UV light  ultraviolet light
XPA  DNA repair protein complementing XP-A cells
XPB  TFIIH basal transcription factor complex helicase XPB subunit
XPC  xeroderma pigmentosum complementation group C, DNA damage recognition protein involved in GGR
XPD  TFIIH basal transcription factor complex helicase XPD subunit
XPF  xeroderma pigmentosum complementation group F
XPG  DNA repair protein complementing XP-G cells
XRCC1  X-ray repair cross-complementing protein 1
1 Ovarian Cancer – a General Introduction

Ovarian cancer (OvC) is malignant neoplastic disease and the most common cause of death from gynecologic cancers, being the fifth leading cause of cancer death in women. The estimated incidence is 15.1 cases per 100 000 women in the Czech Republic (Ferlay et al., 2013) and 6.1 cases per 100 000 women worldwide with nearly 239 000 new cases of OvC diagnosed in 2012 (Sundar et al., 2015). The high mortality of the disease, accounting for almost 152 000 of deaths in 2012 (Ferlay et al., 2015), is associated with the problems with early diagnosis and an initiation of treatment at advanced stages.

Ovarian carcinomas develop for long asymptptomatically or with nonspecific symptoms, the lack of effective and sensitive biomarkers makes the early diagnosis difficult. On the other hand, early start of therapy increases the survival rate and could prevent the spread of cancer to lymphnodes and distant organs (metastases). At early-stage, when the cancer is localized only in the part of the body where it emerged, the 5-years survival among patients with OvC is approximately 92,5 %. In more advanced stages, the 5-years survival rate decreases significantly to 73 % for stage II and III which means, that cancer already spread to surrounding tissue. For stage IV, when the cancer spreads to distant parts of the body, the 5-years survival is only 28,9 % (based on data covering a period 2007-2010) (https://seer.cancer.gov). In this late stage, the complete surgical removal of the tumor in combination with chemotherapy and subsequent recovery is already complicated.

1.1 Histological Features

OvC is a heterogeneous disease which can develop in the ovary’s epithelial, germ or stromal cells. Epithelial ovarian cancer (EOC) represents the predominant part (85 % to 90 %) of all OvCs and is classified in four major histological subtypes – serous cystadenocarcinoma (42 %), clear cell carcinoma (6 %), endometrioid carcinoma (15 %) and mucinous cystadenocarcinoma (12 %). Undifferentiated carcinoma accounts for 17 % of the disease (Di Saia & Craesman, 2012).

1.2 Genetic and Epigenetic Aspects

Family history of OvC and other gynecologic malignancies represents the strongest factor affecting a risk of developing OvC (Stratton et al., 1998). However, only about 10 % of OvC cases are thought to be hereditary, while the remaining 90 % of diagnosed OvC cases are sporadic. Hereditary OvC risk is related to the family history of the disease. On the contrary sporadic cancer means the cancer arising from spontaneous (mainly somatic) mutations on the base of environment and lifestyle interaction. Many high-penetrance susceptibility genes, such as BRCA1 and BRCA2 encoding breast cancer type 1 and 2 susceptibility protein (Rish, 2001), MSH2 encoding mutS homolog 2, MLH1 encoding mutL homolog 1, MSH6 encoding mutS homolog 6 (Bondana et al., 2011), and moderate-penetrance susceptibility genes, such as RAD51C and RAD51D encoding Rad51 paralog C
and D (Song et al., 2015) and BRIP1 encoding BRCA1 interacting protein C-terminal helicase 1 (Rafnar et al., 2011) have been identified in hereditary forms of OvC. Low-penetrance genetic variants are believed to account for remaining excess of relative risk and often have no background in family history of the disease.

Carrying a single-gene mutation may predispose to more than one cancer type. Women with germline mutations in BRCA1 and BRCA2 tumor suppressor genes, which account for about 9/10 hereditary OvC cases (Boyd, 2003), exhibit apart from the high risk of developing OvC also an increased risk of developing breast cancer (Miki et al., 1994; Lancaster et al., 1996). This type of mutations is known as hereditary breast and ovarian cancer syndrome (HBOC). BRCA1/2 are responsible for the repair of DNA double strand breaks (DSBs) using homologous recombination and thus maintain genetic stability of the cell. Mutations in BRCA1, located on chromosome 17q, and BRCA2, located on chromosome 13q, account for the most common cause of hereditary OvC. There is also a tight link between colorectal cancer (CRC) and certain extracolonic cancers such as OvC. Disorder predisposing to these cancer types arise from inherited mismatch repair (MMR) deficiency and is known as Lynch Syndrome (LS) or hereditary nonpolyposis colorectal cancer (HNPCC). Having this condition makes patients unable to repair DNA replication errors, which lead to microsatellite instability (MSI) and subsequent malignant transformation. Individuals with LS have life time risk of developing CRC of about 80%, whereas women with LS have about an 8% life time risk of OvC (Vasen et al., 1996). Even thought LS is genetically heterogenous and is confirmed by mutation in at least one of multiple MMR genes, both MMR-related OvC and CRC occur due to alterations in the same MLH1, MSH2 or MSH6 genes (Ketabi et al., 2012; Barrow et al., 2008).

Epigenetic changes in DNA repair genes play an important role in the onset of sporadic OvC either. These modifications often arise in response to outer or enviromental factors and significantly influence the way how the genes will be transcribed without altering the DNA sequence. Unlike the changes in DNA sequence, epigenetic modifications are frequently reversible and can serve as prospective target of epigenetic therapy (Heerboth et al., 2014). Discovery of epigenetic alternations involved in gene transcription control (such as methylation) (Wei et al., 2006) or in post-transcriptional modifications (such as non-coding RNAs regulation) (Chung et al., 2013) may further contribute to the development of new biomarkers, applicable both in diagnostics and in design of optimized therapeutical regimen. Epigenetic changes are specific for each of four OvC histological subtypes. The most common epigenetic deregulations linked to OvC are aberrant methylation (Catteau et al., 1999), histone modifications (Caslini et al., 2006) and expression of non-coding RNAs (Iorio et al., 2007) regulating gene transcription. When dealing with inappropriate methylation, cytosine-phosphate-guanine (CpG) islands are frequently hypermethylated in OvC as well as in other cancers (Teodoridis et al., 2005). CpG islands are typically located within gene promoters and their hypermethylation often mediates corresponding gene repression. On the other
hand, DNA hypomethylation event affects mostly non-coding DNA, particularly transposable elements and repeat sequences, and has also impact on OvC onset (Widschwendter et al., 2004).

1.3 Risk Factors

The risk of developing OvC grows with increasing age, 45% of all ovarian carcinoma occurs in postmenopausal women older than 65 years (based on data covering a period 2010–2014) (https://seer.cancer.gov). Recent studies show that the use of oral contraceptives can be protective against disease development. This protection becomes more efficient with duration of use (each 5 years of use decrease the risk of OvC by 20%) and has been pronounced among BRCA1/2 mutation carriers (Cibula et al., 2010). As one of the likely explanations seems the effect on number of ovulation cycles. During the ovulation, ovarian surface epithelium undergoes repeated microtraumas. Epithelium becomes liable to faulty DNA replication and thus the probability of cell transformation into cancerous cell increases (Fathalla, 1971). From this reason, the length of menopause (Casagrande et al., 1979), full-term pregnancy (Adami et al., 1994) and breastfeeding (Chiaffarino et al., 2005) might also lower the risk of OvC. Women who have been full-term pregnant have a reduced risk of OvC compared to women who have not given birth. This risk decreases with every single birth at full term. Besides, it has empirically been observed that gynecologic surgical procedures like hysterectomy and tubal ligation also have a beneficial influence on OvC prevention (Hankinson et al., 1993). The involvement of hormones in OvC etiopathogenesis is also significant. According to several theories, mitigation of gonadotrophin levels and estrogens along with elevated progesterone have an influence on cancer risk reduction (Vanderhyden, 2005).

1.4 Treatment

A frequent problem related to the OvC treatment is developing drug resistance in patients who were initially responsive to chemotherapy. As therapeutic agents, paclitaxel and carboplatin are commonly used. Acquired resistance can result from an activation of energy dependent transport proteins (Samimi et al., 2004), known as efflux pumps, that are after their overexpression able to detect and exclude the xenobiotics or their metabolites from the cell. Among these proteins, P-glycoprotein 1 (P-gp), ATP-binding cassette (ABC) transporter with broad specificity, is of importance (Bell et al., 1985). Additionally, secondary somatic mutations in OvC relevant genes such as BRCA1/2 (Norquist et al., 2011) may underlie the onset of the resistance towards chemotherapeutics. Epigenetic therapy in combination with some chemotherapeutic agents poses an option in chemotherapeutic sensitivity restoration (Matei et al., 2012). Apoptotic pathways activation is another treatment approach in OvC (Sasaki et al., 2000). Due to the overexpression of some anti-apoptosis factors and executional caspases inhibition, fraction of the OvC cell become resistant to chemotherapeutics and subsequent therapy loses its efficiency. B-cell lymphoma 2 (BCL-2)
and tumor suppressor protein p53 (p53) family of apoptosis regulator proteins (Eliopoulos et al., 1995) and phosphatidylinositol-4,5-bisphosphate 3-kinase / AKT serine/threonine kinase (PI3K/Akt) pathway (Lee et al., 2004) are potential candidates for resistance adjustment.

2 What is DNA Repair and Why is Important; a) in General and b) in Relation to Ovarian Cancer Etiology

DNA repair is the biological process by which the cell recognizes and corrects damaged DNA. This mechanism is essential for the long life span of the cell and preventing error accumulation. In case that these repair processes fail and the cell does not undergo apoptosis or senescence, DNA lesions persist in the organism and may be transmitted from the one cell generation to the other. As a last resort, accumulated mutations contribute to carcinogenic process.

Seven major types of DNA repair pathways are implicated in DNA damage response (DDR). Arising DNA DSBs can be repaired by homologous recombination (HR), non-homologous end joining (NHEJ) or microhomology-mediated end joining (MMEJ). By contrast, single strand breaks (SSBs) are repaired by mechanisms including base excision repair (BER), nucleotide excision repair (NER) and mismatch repair. Direct reversal of DNA damage makes another possibility to fix DNA strands. SSBs arise in cells from attack of reactive oxygen species, intracellular metabolites, from the intrinsic DNA instability and also as DNA repair intermediates, whereas ionizing radiation, reactive free radicals and recombination across a nick predominantly account for formation of DSBs. Additionally, some DSBs and SSBs are not formed directly, but as a consequence of the attempted repair by BER. This potential damage is associated with UV exposure, bulky lesions and platinated derivates. In context of OvC, four major types of DNA repair such as HR, NER, BER and MMR have been reported.

Faulty DNA repair pathways pose a risk especially if unrepaired DNA damage occurs in tumor supressor genes (TSGs) or proto-oncogenes. In both instances, it results in non-controlled cell proliferation. Loss of tumor suppresor functions and activation of proto-oncogene usually arise from deletion, epigenetic silencing and mutation of germline or somatic origin. In epithelial OvC, somatic variants of mutation predominate notably over these with hereditary character. The prevalence of OvC cases arises from somatic alterations (in more than 90 %), whereas remaining less than 10 % of cases are associated with germline mutations (Narod et al., 1994).

2.1 Tumor Suppressor Genes

Tumor suppressor genes are negative regulators, which coordinate cell division by stopping or slowing its proliferation during the mitotic phase G1. Mutations in tumor suppressor genes have
usually recessive character and can be hereditary as well as somatic origin. In the former case, one defective allele is inherited and the disease develops later by somatic inactivation of the second allele. In the latter case, the loss of both alleles happens through somatic mutation. Major tumor suppressor genes associated with epithelial ovarian cancer encode BRCA1 (Miki et al., 1994), BRCA2 (Lancaster et al., 1996), p53 (Marks et al., 1991), phosphate and tensin homolog (PTEN) (Saito et al., 2000), caveolin 1 (CAV1) (Miotti et al., 2005) and retinoblastoma protein (pRb) (Li et al., 1991). All these genes are required for DDR – to find DNA lesion and through the signalization promote its repair. Obviously, their defect leads to loss of control over the DNA damage. Cells with disabled tumor suppressor genes manifest sensitivity to DNA damaging agents as well as predisposition to disease development.

2.2 Proto-oncogenes

By contrast, proto-oncogenes stimulate the cell growth and govern its proliferation in each of the checkpoints. Through mutation, they change to oncogenes. Since the proto-oncogene is transformed into oncogene, it can not restrain cell division and cell can grow out of control. Fortunately, single mutated oncogene is still usually insufficient to start tumorigenesis assuming the proper function of tumor suppressor genes. Pivotal proto-oncogenes involved in ovarian cancer are v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) (Mok et al., 1993), myc proto-oncogene protein (c-myc) (Baker et al., 1990), AKT serine/threonine kinase 1 (AKT1), AKT serine/threonine kinase 2 (AKT2) (Altomare et al., 2004) and human epidermal growth factor receptor 2 (HER-2/neu) (Berchuck et al., 1990).

3 DNA Repair Pathways

3.1 Homologous Recombination Repair

Homologous recombination (HR) refers to several processes by which a cell exchange similar or identical DNA segments between the two strands. This mechanism occurs not only in meiotic prophase I to create genetic variation, but it is also applied in repairing DSBs, which are extremely harmful types of DNA lesions. The programmed formation of DSBs is induced mainly during meiosis, to carry out genetic exchange between homologous chromosomes. Common environmental damage, in which HR is employed, includes radiation (Corry & Cole, 1968), UV light (Covo et al., 2012) and crosslinking agents (McHugh et al., 2000). In this instance, DSBs arise indirectly as an intermediate after the cross-link removal.
3.1.1 Mechanism

Homologous recombination repair (HRR) (Fig. 1 on page 7) is initiated by MRN complex, a heterotrimeric protein consisting of double-strand break repair protein Mre11, DNA repair protein RAD50 and protein NBS1, which binds to DSB (de Jager et al., 2001). Mre11 then catalyses the short-range resection of 5´ends at the break site (Paull & Gellert, 1998). Although generated 3´ tails are usually sufficient to promote DSB repair, either exonuclease 1 (EXO1) (Tran et al., 2002) or RecQ helicases together with DNA2 helicase (Sturzenegger et al., 2014) ordinarily continue processing these intermediates to produce a long overhanging 3´ tails. In this stage, replication protein A (RPA) binds to exposed single-stranded DNA (ssDNA) and prevents the formation of secondary structures (Pestryakov et al., 2003), because ssDNA serves as a substrate for RAD51 protein (Ma et al., 2016). RAD51 assembly is mediated primarily by BRCA2 (A. A. Davies et al., 2001), however in case of BRCA2 deficiency, RAD52 DNA repair protein (RAD52) may supply its function (Feng et al., 2011). BRCA1 also interacts with RAD51 and its function seems to be similar to BRCA2 (Scully et al., 1997). When RAD51 is loaded onto the ssDNA, it searches for sequence homology and facilitates the pairing between 3’ssDNA overhang strand and undamaged homologous duplex (Baumann et al., 1996). During homologous exchange, invading strands form a complex called displacement loop (D-loop), which consist of new heteroduplex and the displaced strand originating from former duplex. In the next step, new nucleotides are synthetized by polymerase from 3´ends and joined together with the second DSB ends. This intermediate is known as holliday junction (HJ). Resolution of the HJ by nicking two strands (Constantinou et al., 2001) results in recombinant crossover or non-crossover double-stranded DNA (dsDNA) as a final product. Nevertheless, during mitosis the DSB repair product is most likely non-crossover and it is provided by synthesis-dependent strand annealing (SDSA) HR-mediated pathway (Stark & Jasin, 2003). On the contrary, during the meiosis another HR-mediated pathway called double strand break repair (DSBR) can lead either to crossover or non-crossover. The choice between these two options relies on the orientation of HJ cutting, especially since double holliday junction (DHJ) is formed in this repair model (Allers & Lichten, 2001). In SDSA, single HJ is the only intermediate.
3.1.2 Links between Homologous Recombination and Cancer

DNA repair by HR is essential for the accurate repair of DSBs. Thus, genetic elements or cellular events that turn off HRR result in high frequency of mutations within the genome and potential cancer development. Impaired HR is responsible for several cancer phenotypes,
especially ovarian and breast cancer. In OvC cell, HR deregulation is driven mostly by somatic and germline mutations in BRCA1/2 (Moynahan, 1999; Moynahan et al., 2001). Less common defects occur in core RAD genes RAD51 and RAD52 (Tong et al., 2003), CDK12 (Ekumi et al., 2015) and other HR DNA damage genes. BRCA1 and RAD51C promoter methylations were also described in this connection (Baldwin et al., 2000; Meindl et al., 2010). When HRR apparatus fails, the cell still have two alternative DSBs repair pathways: NHEJ and MMEJ. Current studies indicate that NHEJ suppression occurs besides other things also in context with BRCA1 mutation (Bau et al., 2006). In contrast to BRCA1, BRCA2 status have been demonstrated to affect only HRR (Xia et al., 2001).

### 3.1.2.1 DNA Polymerase Theta and Its Role in Homologous Recombination Repair

DNA polymerase theta (pol Θ) upregulation is frequently reported in ovarian carcinoma. Pol Θ is a family A DNA polymerase involved in DSBs repair. It is an error-prone enzyme encoded in humans by the POLQ gene. Its very low fidelity comes from the lack of proofreading activity and error rate, which is more than 10-fold higher than for other two human proofreading-deficient A family polymerases (Arana et al., 2008). Human pol Θ has moderate processivity and synthesizes chains that differ in length from 1 to more than -75 nucleotides (Arana et al., 2008). Pol Θ has highly conserved structure among multicellular organisms and it is made up of four subunits. N-terminus forms a helicase-like domain, comprising several structural motifs characteristic for DNA and RNA helicases. The central domain contains RAD51 interacting motif, while C-terminal domain serves as the polymerase activity hub. It was proved that the expression of pol Θ inversely correlates with expression HR genes and inhibits HRR pathway (Ceccaldi et al., 2015). This inhibition is realized by pol Θ ability to bind RAD51 recombinase, thus pol Θ prevents the RAD51 assembly on ssDNA binding proteins and blocks RAD51-mediated HR. On the basis of cell’s ability to carry out HR, different situations may occur. In the first case, lowering the pol Θ expression in HR-proficient cells has the consequence in HR activity upregulation. While in the cancer cells defective in HR and incapable of pol Θ production, the loss of pol Θ increases cell death. HR-deficient cells with upregulated pol Θ expression are observed in various cancers, most frequently in ovarian and hereditary breast cancer. It was found out that in epithelial ovarian cancers, pol Θ expression has upward trend depending on tumour grade (Ceccaldi et al., 2015). From the studies mentioned above it may be deduced that by reducing the pol Θ synthesis, cancer cells become more sensitive to anti-cancer drugs and radiation. This finding could therefore have a potential use for new cancer therapy targeting at pol Θ knockdown. According to these results, pol Θ seems to be also a prospective biomarker in cancer prognosis.

### 3.1.2.2 Association of Human RAD51 Protein with Ovarian Cancer Risk

Human RAD51 protein is also connected with OvC risk. The main importance of RAD51 is providing DNA pairing and strand exchange. RAD51 protein family is known to be cooperating
with BRCA2. Their interaction appears to be crucial mainly because of the fact that after initial end resection step of HRR, BRCA2 promotes RAD51 targeting to newly emerged 3´tailed DNA and its assembly on it. Under normal conditions, BRCA2 binds RAD51 with about six RAD51 molecules per one of BRCA2, where the number of RAD51 bound grows up linearly with its concentration and is dependent on content of BRC repeats in BRCA2. With regard to DNA binding affinity of BRCA2, ssDNA substrate, especially tailed ssDNA generated on DNA duplex, is strongly preferred as compared to dsDNA. Thus the presence of BRCA2 is also required for prevention of RAD51 binding to dsDNA (Jensen et al., 2010). Among other things, BRCA2 maintains DNA strand exchange in a concentration dependent manner using ATP for energy. During its loading onto ssDNA, RAD51 competes for the same binding sites with RPA and displaces it due to its tight bonds with DNA very slowly. RPA makes complex with ssDNA and keeps DNA strands unwound for replication, initial phase of HR or nucleotide excision repair process. Increasing amount of BRCA2 supresses the inhibition caused by RPA and stimulates DNA strand exchange (Jensen et al., 2010).

RAD51 protein family also fix DNA crosslinks induced by various reactive groups or radiation agents. Two kinds of these lesions can arise. Intrastrand crosslinks occur by covalent connection between bases in the same DNA strands, while interstrand crosslinks (ICLs) occur between residues in the opposite strands. DNA modified in this way poses a serious threat because crosslinking of DNA forms an obstacle to the separation of complementary strands. Thereby DNA replication and transcription is potently inhibited. RAD51 paralogs form an BCDX2 complex, which is implicated along with HELQ helicase (HELQ) in ICL repair pathway. Another complex made up of RAD51 paralogs, CX3 complex, does not tightly participate. HELQ performs its role by unwinding DNA strands at blocked replication fork and acts directly with BCDX2. Because of this fact, HELQ and BCDX2 show themselves as the indispensable elements of the whole HRR process and affect sensitivity to ICLs. Experiments have demonstrated that humans with deficiency of HELQ are predisposed to ovarian and pituitary tumorigenesis. Fertility defects linked to smaller genitalia and germ cell depletion and more frequent tendency to form ICL have been reported as well (Adelman et al., 2013). All in all, individuals with HELQ deficiency have the similar phenotype like these with Fanconi anemia (FA) disease. Following experiments authenticate that both pathways are mutually independent, where HELQ corresponds to Fanconi anemia complementation group D 2 protein (FANCD2) in FA pathway (Adelman et al., 2013). Cells with silenced HELQ genes seem to be sensitive to cisplatin and mitomycin C (MMC), other tested DNA-damaging agents are not effective permanently (Takata et al., 2013). With regard to all these facts mentioned above, HELQ gene may be potential genetic marker for OvC diagnosis.
3.2 Mismatch Repair

DNA mismatch repair is a system that ensure error recognition and correction processes during DNA replication and recombination. During DNA replication, it maintains removal and fixing erroneously incorporated bases, that have escaped DNA polymerase proofreading, using parental strand as a template. The same MMR machinery proceeds mismatched nucleotids in heteroduplex formed during homologous recombination. In contrast, gene conversion between divergent sequences is intensively inhibited by MRR proteins.

3.2.1 Mechanism

The human MMR system (Fig. 2 on page 11) is ensured by the Mut proteins, namely by methyl-directed mismatch repair proteins MutS (MutS) and MutL (MutL) homologous to the bacteria Mut proteins. MutS is a heterodimeric ATPase constituted of MSH2/MSH6 (MutSa) or MSH2/MutS homolog 3 (MSH3) (MutSβ) subunits (Obmolova et al., 2000), just as MutL constituted of MLH1/PMS1 homolog 2 (PMS2) (forming MutLα), MLH1/PMS1 (forming MutLβ) or MLH1/Mutl homolog 3 (MLH3) (forming MutLγ) (Kadyrov et al., 2006; Räschle et al., 1999; Cannavo et al., 2005). The initial step of base-base mismatch recognition is provided by MutS, which binds to DNA near the mismatch site (Tessmer et al., 2008) and from afar communicates with methyl-directed mismatch repair MutH (MutH). Meanwhile, MutH searches hemimethylated dGATC sites in daughter DNA and when identifies such site, it induces DNA bending (Längle-Rouault et al., 1987). The task of another MMR protein MutL is to coordinate individual MMR components (Ban & Yang, 1998). First of all, MutL joins the MutS-DNA complex and via MutS mediates MutH activation. This event allows MutH to generate nick near the hemimethylated site. At the same time, one of the ssDNA endonucleases, depending on the break position relative to the mismatch, is activated by MutS (Genschel & Modrich, 2003) and degrades arising DNA strand as it becomes unwound. In the distance of about 100 bp beyond the mismatch, MutL terminates endonuclease activity and nascent ssDNA is rapidly occupied by SSB proteins, which prevent formation of DNA secondary structure as well as protect ssDNA from being cleaved by endonuclease. In the presence of the proliferating cell nuclear antigen (PCNA) DNA clamp and replication factor C (RFC), DNA polymerase delta (pol δ) then adds new bases until the gap is completely filled. Ultimately, DNA ligase 1 (LIG1) is employed to seal the nick and MMR is completed (Zhang et al., 2005).
3.2.2 Links between Mismatch Repair and Cancer

Defect of any kind of Mut proteins may result in microsatellite instability (MSI), pathogenic gene conversion and in the worst case also multistep tumorigenesis (Xue et al., 2014). Microsatellites are fragments of repetitive DNA (mainly adenine (A) or adenine-thymine (AT)), they come into existence by replication slippage while the daughter strand of DNA is forming. Replication slippage often occurs in tandem repeat sequences which involves DNA polymerase to separate from the template. As follows, incomplete daughter strand strand slips out from it and pairs with another direct repeat in the 3’ to 5’ direction. DNA polymerase then reattaches to the daughter strand upstream and continues to copy the template. However, as a consequence of the new strand pairing, DNA loop on one of the strands is generated. If the loop is formed on daughter strand, extra nucleotides are
incorporated because DNA polymerase inserts the same nucleotides already added before. If the template loops out, DNA polymerase skips over one or more nucleotides and the deletion is introduced. These incorrect sequences can be fixed easily if operated under normal circumstances. However, impaired MMR can not cope with these errors, which often results in frame-shift mutations. When a frame-shift occurs in cancer-related genes, it can induce loss of their function that ultimately leads to cancer.

MMR deficiency is the whole phenotype in CRC and its hereditary form, LS (HNPCC), is crystallographic example. This is also the reason why deficiencies in MMR predispose patients to get as a second cancer OvC (in case of CRC) or CRC in OvC patients. MMR deficiency affects OvC etiology from about 10% to 15% (Bewtra et al., 1992).

MMR dysfunction may have both genetic and epigenetic origin. Mutations in DNA MMR genes account for the most cases of hereditary OvC excluding BRCA1 and BRCA2 gene defects. Inherited MMR deficiency is frequently associated with LS, formerly known as HNPCC. LS is an autosomal dominant genetic condition caused by defective MMR proteins, mostly by MSH2 and MLH1 (Dowty et al., 2013). Patients with LS face high predispositions to CRC and also increased likelihood of developing endometrial, ovarian, stomach, small intestine and numerous different types of cancer (Watson et al., 2008). LS-associated ovarian cancer subtypes are very variable, mixed endometrioid/clear cell type prevails. Mutations in MSH2 affect 47% and in MLH1 38% of women (Helder-Woolderink et al., 2016). Simultaneous mutation on both BRCA1/2 and MMR genes is a rare event. In this context, co-occurrence of BRCA1 and MSH6 truncation mutations was reported in patients with endometrial cancer (EC) (Kast et al., 2012).

At present, genetic MMR disorders such as Lynch-like syndrome (LLS), sporadic MSI CRC cancer and constitutional MMR deficiency (CMMRD) also come under the umbrella of HNPCC. Patients with LLS have seemingly the same symptoms as those with LS. They manifest high MSI and lack MMR proteins. Unlike them, however, LLS patients do not exhibit germline mutations or methylations in MMR genes (Rodriguez-Soler et al., 2013). Exact mechanism of MMR inactivation is still under investigation.

As for other mentioned disorders, sporadic MSI CRC makes about 15% of all sporadic CRC cases (Hampel et al., 2005). It stems mostly from a biallelic hypermethylation of MLH1 promoter, BRAF(V600E) proto-oncogene mutation is a common accompanying phenomenon (Vilkin et al., 2009). The last-named CMMRD is highly penetrant syndrome which occurs rarely due to biallelic germline mutations in MMR genes (Bakry et al., 2014).

Distinguishing between the particular MMR disorders is important because of their different familiar predispositions and colorectal and associated extracolonic cancers incidence. Increased personal chance of getting second primary cancer, such as OvC, applies to all HNPCCs.
3.3 Nucleotide Excision Repair

Nucleotide excision repair identifies and removes DNA helix-distorting lesions on ssDNA. In contrast to BER, NER recognizes general damage to DNA, such as bulky DNA adducts. Chemicals that form these alternations are mostly of exogenous origin and include especially products of oxidative metabolism (Reardon et al., 1997) and many food carcinogens, such as polycyclic aromatic hydrocarbons (Braithwaite et al., 1998), heterocyclic aromatic amines (Reeves et al., 2011), heterocyclic amines (Felton et al., 2007) etc. From physical agents, UV radiation (Muñoz et al., 2017) should not be forgotten.

3.3.1 Mechanism

NER (Fig. 3 on page 14) can be divided into two general subpathways: transcription coupled NER (TCR) (Mellon et al., 1987), removing the lesions created on primary transcript during the gene transcription, and global genomic NER (GGR) (Yu et al., 2016), removing the lesions occurring in the entire genome including the non-transcribed genes on DNA strand currently undergoing transcription. Both of the subpathways share the same repair mechanism but differ in early damage recognition step. In GGR, xeroderma pigmentosum complementation group C (XPC) complexing with RAD23B homolog B (RAD23B) and heterodimer consisting of DNA damage-binding protein 1 and 2 (DDB1/2) are required for recognising DNA damage. XPC/RAD23B is responsible for recognising distorting lesions (Kusumoto et al., 2001) whereas DDB1/2 identifies damage caused by UV light (Wakasugi et al., 2002). As for TCR, a DNA lesion stops replication fork and damage is recognized directly by RNA polymerase II (RNAP II) cooperating with Cockayne syndrome A (CSA) and B (CSB) proteins. Proper GGR function is tightly linked to TP53 tumor-suppressor gene encoding p53 protein. If the p53 is not produced, GGR is interrupted. The following studies also reported that BRCA1 promotes GGR capacity independently on p53, when activates XPC, DDB2 and GADD45 gene expressions required for NER (Hartman & Ford, 2002). In TCR, BRCA1 involvement remains inconclusive.

The second step in NER process is damage verification. It is maintained by transcription factor II human (TFIIH), large multisubunit protein composed of ten subunits - TFIIH basal transcription factor complex helicase XPD subunit (XPD) (Shaeffer et al., 1994) and TFIIH basal transcription factor complex helicase XPB subunit (XPB) (Hwang et al., 1996), CDK-activating kinase complex (CAK) (Shiekhattar et al., 1995) and several proteins including transcriptional regulator p8 (p8), protein kinase p34 (p34), protein activator p44 (p44), matrix protein p52 (p52) and ubiquitin-binding protein p62 (p62) (Bedez et al., 2013). XPD and XPB unwind DNA and all the complex stops in damaged site (Evans et al., 1997). When the presence of the lesion is confirmed, DNA repair protein complementing XP-G cells (XPG), which have the 3’-endonuclease activity (O’Donovan et al., 1994) or excision repair cross-complementation group 1- xeroderma pigmentosum
complementation group F (ERCC1-XPF) enzyme complex, which have the 5’-endonuclease activity (Sijbers et al., 1996), join the TFI IH. In addition, RPA and DNA repair protein complementing XP-A cells (XPA) repair proteins (Vasquez et al., 2002) attach TFI IH and promote its interaction with DNA. In this point, preincision complex, which assembly is provided by XPD-DNA interaction (Vashisht et al., 2015), is formed and endonuclease cleaves damaged DNA fragment. Resulting gap is filled by pol δ or by polymerase epsilon (pol ε), copying the undamaged DNA strand, in cooperation with PCNA, RFC and RPA – the same protein machinery required for DNA replication synthesis (Shivji et al., 1995; Overmeer et al., 2010). In the last stage of NER process, DNA nick is sealed either by LIG3-ERCC1 complex (Kusumoto et al., 2001) or by LIG1, which exclusively serves in TCR (Levin et al., 1997).

![Fig. 3 Nucleotide excision repair. General scheme (adapted from Kimura & Sakaguchi, 2006)](image)

**3.3.2 Links between Nucleotide Excision Repair and Cancer**

Under physiological conditions, NER is essential to protect the cell against the mutagenesis and beginning of carcinogenesis (an initiation phase). Conversely, in some kind of cancer treatment...
such as chemotherapy, loss of nucleotide excision repair capacity is even desired. It is because chemotherapeutics, such as overwhelming majority of alkylating and platinum-based agents, introduce bulky DNA lesion causing replication arrest and subsequent apoptosis, if NER apparatus fails. However, repair of DNA damage induced by methylating agents is almost always reliant on BER (Fishel et al., 2007).

A textbook example of OvC treatment that cause DNA damage is widely used cisplatin, cross-linking agent with chemical formula cis-[PtCl\(_2\)(NH\(_3\))\(_2\)]. When getting inside the cell, one of its chlorides is displaced by water molecule. By this way, cisplatin converts into its active form and becomes more electrophilic. Bases on DNA strand, typically guanine, binds to the complex, because nitrogen-7 position of guanine is a strong nucleophile and can be easily attacked by cisplatin and causes its dehydratation. Nitrogen-7 of adenine and also nitrogen-3 of cytosine less commonly bind to cisplatin, but they are not too reactive toward it. When the bond between the base and cisplatin is formed, remaining chloride of cisplatin is then displaced usually by another guanine and DNA crosslink is established. Intrastrand cross-links constitutes 90% of all crosslinks and are formed between two adjacent bases (1,2-d(GpG) or 1,2-d(ApG)), remaining cross-links are interstrand (1,2-d(GpC)) or intranstrand between the purines separated by another base (1,3-d(GpNpG)). Impact of these harmful lesions depends mainly on NER, because it is responsible for their removal. As follows, NER is replaced by HRR which is applied to fix DSBs repair intermediate left by exonuclease or endonuclease activity within NER.

Partly due to enhanced NER (besides the activity of ABC and solute carrier (SLC) membrane transporters etc.), rapid development of drug resistance often occurs and complicates the effect of chemotherapy treatment. An interesting solution for dealing with this problem is a suppression of NER making OvC cells to be more sensitive to DNA damage agents.

Undoubtedly the most frequently mentioned NER protein when speaking of platinum resistance in OvC is ERCC1. This protein is required for NER, ICL repair and DSBs repair. Its increased expression is verifiably linked to more efficient NER and correlates with reduced sensitivity to cisplatin. Subsequent research confirmed that targeting of ERCC1 by antisence RNA improves the effectiveness of chemotherapy and suppresses NER activity (Ferry et al., 2000; Selvakumaran et al., 2003). Moreover, level of its expression can be useful for predicting platinum-based chemotherapy resistance.

3.4 Base Excision Repair

Base excision repair ensures removing specific small lesion from ssDNA and protects DNA from endogenous and exogenous small molecular mutagens. Such DNA lesions do not usually distort helix structure and are caused mainly by alkylation (Lau et al., 1998), oxidation (Rusyn et al., 2004), deamination (Cortellino et al., 2011) and uracil misincorporation (Nilsen et al., 2002).
3.4.1 Mechanism

Eleven damage-specific DNA glycosylases play a key role in human BER (Fig. 4 on page 17) process. This family of enzymes cleaves modified or misincorporated bases from DNA leaving apurinic/apyrimidinic (AP) sites in DNA structure (Jacobs & Schär, 2012). Some glycosylases such as endonucleases VII-like 1 (NEIL1) and 2 (NEIL2) have also lyase activity and in the next step cleave DNA backbone and remove 3´-α,β-unsatured aldehyde group. Phosphate retained at the 3´ terminus is then removed by polynucleotide kinase 3´phosphatase (PNKP) (Wiederhold et al., 2004), in order to expose 3´OH group. On the other hand, monofunctional glycosylase are not able to form SSB and this task including all 3´ blocking groups removal have to be fulfilled by AP endonuclease 1 (APE1), which have also the diesterase activity (Suh et al., 1997). Although the 3´OH terminus is free, at the 5´ terminus remaining deoxyribophosphate (dRP) has to be cut out by DNA polymerase β (pol β) (Matsumoto & Kim, 1995). Exposed 3´OH group enables polymerase to fill the gap by newly synthesized nucleotide and the repair is completed with nick sealing catalysed by DNA ligase 3 (LIG3) in complex with the X-ray repair cross-complementing protein 1 (XRCC1) (Abdou et al., 2015). According to the number of removed nucleotides and also according to oxidation state of abasic sugar, nucleotide synthesis is processed either by short-patch BER (described above) or long-patch BER (Sattler et al., 2003). If only one nucleotide is needed, short-patch BER is favoured, whereas synthesis of two up to ten nucleotides is within long-patch BER. Furthermore, pol β is only polymerase implicated in short-patch BER (Singhal & Wilson, 1993), whereas in long-patch BER besides pol β (Dianov et al., 1999) there are also pol δ and pol ε involved (Stucki et al., 1998). Pol β lacks the ability to cut oxidized or reduced 5´sugars out and that is the reason, why in such cases long-patch BER is preferred to short-patch BER. In long-patch BER, pol β, δ or ε, in presence of PCNA and RFC, displace modified sugar and form the 5´ flap intermediate, which is then resolved by flap endonuclease 1 (FEN-1) (Klungland & Lindhal, 1997) and sealed by DNA LIG1 (Sleeth et al., 2004).
3.4.2 Links between Base Excision Repair and Cancer

DNA repair deregulation, including BER, leads to genome instability and often results in genetic diseases or oncogenesis initiation. Upregulation of pol β appears to be frequently reported in connection with human cancers including OvC (Bergoglio et al., 2001), e.g. in KRAS(G12C)-mutated OvC cells (Caiola et al., 2015). These data also suggest that pol β activity may support achieving drug resistance phenotype via enhanced NER.
Variants in MutY DNA glycosylase (MUTYH), required for excising oxidative DNA damage, cause MUTYH-associated polyposis (MAP). This autosomal recessive syndrome is chiefly attributable to an increased risk of CRC (Win et al., 2014) and moderately also to an OvC risk (Vogt et al., 2009). Regarding OvC, low expression of the MBD4 gene, encoding methyl-CpG-binding domain protein 4 (MBD4), stemmed from its promoter methylation, was described in OvC and CRC (Howard et al., 2009). MBD4 is DNA glycosylase, which targets spontaneously deaminated bases and also binds by methyl-CpG binding domain to methylated DNA. Its downregulation results in MSI, decreased programmed cell death and mutations occurring more frequently in CpG islands.

Several single nucleotide polymorphisms (SNPs) were also found in OGG1 and APEX genes in ovarian and endometrial tumors (Pieretti et al., 2001). OGG1 encodes 8-oxoguanine glycosylase removing 8-oxoguanine DNA damage caused by oxidative stress. APEX encodes APE1 important in the process of single 5′DNA cleavage. However, somatic mutations in these genes were not identified. SNPs located in genes responsible for maintaining BER seem to be associated with genetic carriers of BRCA1 and BRCA2 mutations. The strongest relationship proved to exist between SNP rs1466785 in the NEIL2 gene and BRCA2 associated breast cancer risk, and between SNP rs2304277 in the OGG1 gene and BRCA1 associated ovarian cancer risk (Osorio et al., 2014). Targeting the cellular DNA repair system in order to improve the chemotherapy treatment is a common tool to fight cancer. To enhance the effect of alkylating agents using in OvC chemotherapy, BER regulation via one of the BER components, N-methylpurine DNA glycosylase (MPG), can be performed (Fishel et al., 2007). This enzyme catalyzes the cleavage of alkylated and deaminated bases. A way to make the cell to be more sensitive to these types of lesions is to disrupt MPG repair mechanism by addition of some synthetic inhibitor such methoxyamine. This small molecule irreversibly inserts into a DNA-AP sites generated by MPG and prevents BER to be completed. Formation of such blocked AP sites leads to consequentially increased presence of SSBs and DSBs and ultimate apoptosis.

Another approach to affect BRCA1/2-defective or in another manner HR-defective OvC cells is poly-ADP ribose polymerase (PARP) inactivation. This BER protein repairs SSB. When it fails, discontinuous DNA strands persist in the cell and during the replication, the DSBs can be easily formed. In normal cells, repair of DSBs is operated via HR. However in BRCA1/2-deficient cells, HR function is interrupted and its impairment is critical for cells survival (Ledermann et al., 2016).

4 DNA Repair and its Role in Ovarian Cancer Therapy

There is a variability of treatment regimens. The medical procedure is recommended according to stage of the disease and the cancer subtype. Advanced OvC is most successfully treated by surgery with subsequent chemotherapy (Griffiths et al., 1979). Other options including hormone (Yokoyama & Mizunuma, 2013), radiation (Martinez et al., 1985) or targeted therapy (Yap et al., 2009) are used
as well. Therapy strategies that take into account particular cancer-critical genes make another interesting method of treatment.

Nevertheless, despite the initial good response to treatment, high percentage of patients experience cancer recurrence within two years from the start of the therapy. Cancer relapse depends on a variety of reasons such as tumor staging and grading, and the presence of remaining malignant tissue after debulking surgery (Rubin et al., 1991).

4.1 Chemotherapy

Chemotherapy for OvC is often based on a combination of two or more drugs. Response to the first-line therapy is usually very high, but the cells quickly acquire a chemoresistance. Platinum compounds, including carboplatin or cisplatin, combined with taxanes, such as paclitaxel and docetaxel, prove to be similarly efficient in first-line therapy (du Bois et al., 2003; Vasey et al., 2004). However, carboplatin together with paclitaxel or docetaxel is mildly preferred because of the lower toxicity and better tolerability compared to cisplatin. These findings make platinum based chemotherapy a cornerstone of OvC treatment.

If the first line-therapy is not efficient, second-line therapy is initiated. Second-line treatment is chosen in agreement with molecular basis of particular chemoresistance and also with regard to length of platinum-free interval (PFI) (Markman et al., 1991), which is defined as the time span between the completion of first-line therapy and the detection of relapse. Another criteria deemed important are drug toxicity and overall impact of treatment on health-related quality of life (Chase & Wenzel, 2011). According to PFI duration, patients can be separated into four categories as follows: a) platinum refractory (with disease progressing during the last line therapy or within four weeks after the end of the therapy), b) platinum resistant (with disease progressing between one and six months), c) potentially platinum sensitive (with disease progressing between six months and one year) and d) platinum sensitive (with disease progressing later than one year). Even though resistance to platinum changes over time, the response rate in second round is usually lower than that in initial chemotherapy. Platinum refractory or platinum resistant tumors are mostly treated by non-platinum drugs, namely by bevacizumab (Cannistra et al., 2007), liposomal doxorubicin (Muggia et al., 1997), gemcitabine (Friedlander et al., 1998), topotecan (Creemers et al., 1996) etc., whereas potentially platinum sensitive and platinum sensitive malignancies are usually anew expose to platinum. Singleplatinum agents such as carboplatin are often used (Bolis et al., 2001).

4.2 Hormone Therapy

OvC cells often contain elevated level of hormone receptors. Because of that, exposure to estrogens may promote their growth and proliferation, whereas progesterone and syntetic progestins have the exact opposite effect. Drugs able to block hormone receptors represent another possibility
to fight cancer. Good clinical results were achieved by treating with tamoxifen medication which binds competitively to the estrogen receptors (Karagol, 2007). It can however induce DNA adducts which are undesirable (Schild et al., 2003). Inhibitors targeting aromatase, which participates in estrogen synthesis, were also proved to be helpful in ovarian cancer treatment (Smyth et al., 2007). Nevertheless, hormone therapy serves mainly as additional treatment and its effect is not very significant.

4.3 Radiation Therapy

OvC radiotherapy is currently rare and in the most cases serves as complementary treatment in selected spots, where the cancer appears (Dembo, 1992). OvC cells with BRCA1/2 mutation evince high sensitivity to ionizing radiation (Yuan et al., 1999) and are suitable for radiation therapy regime.

4.4 Targeted Therapy

Targeted therapy represents modern approach selectively affecting cancerous cells with minimal impact to healthy cells. Drugs used in this type of therapy are mostly designed to focus on cell mechanisms different from their physiological state. The usual way of providing targeted therapy is switching of chemical signalization to cell division (Cao et al., 2006), stopping intensified angiogenesis essential for cell nutrition (Spannuth et al., 2008), blocking repair pathway leading to apoptosis (Hellday et al., 2008) or blocking gene expression, e.g. by histone deacetylase inhibitors (Qian et al., 2006). Histone deacetylases remove acetyl groups from the lysine ε-amino groups on a histone, thereby change the positive charge of the histone to negative and enable DNA to be more accessible to transcription factors. Last but not least, immune system (Preston et al., 2011) or toxin transport to cancerous cell (Shapira & Benhar, 2010) are currently exploited. Great sensitivity compared to high chemotherapeutic cytotoxicity and therapeutic effect only in desired site makes targeted therapy very attractive for future medical treatment. In this review, DNA repair and its regulation has been analysed in detail. Anticancer therapy focus on DNA repair pathways particular due to the fact that enable cells to survive DNA lesions induced by chemotherapeutics. For that reason, inhibition of specific DNA repair pathways contributes to better response to chemotherapy and other therapies relied on induction of DNA damage. Currently used therapies evoke DNA damage e.g. by disruption in nucleotide metabolism (Aird et al., 2014), polymerase inhibition (Ledermann et al., 2012), inhibition of DNA damage sensors (Huntoon et al., 2013) etc. Furthermore, some deficiencies induced in DNA repair pathways lead to their malfunction and the cell is forced to use alternative repair pathway (if available) (Fong et al., 2010). The problem lies in compromised DNA repair ability in some cancer types. If the cancer cells have such alternative pathways impaired, it implicates accumulation of cytotoxic lesions, which can not be removed, and leads mostly to apoptosis.
5 Conclusion

This thesis summed up the most apparent molecular alternations observed in OvC tumors, which often elicit a reduced ability to repair DNA damage and deregulation of cell growth. The involvement of the DNA repair pathways in cancer treatment have also been discussed. Since the DNA repair machinery is well-known, insight into its function allows therapies targeting the components of a particular repair pathway. DNA repair manipulation has a great potential, because throught its modulation, pro-apoptotic effect of chemotherapeutic agents can be enhanced.

DNA repair genes reported in the context of OvC may help with cancer diagnosis and determination of genetic predisposition to the disease. Many cancer screening test are currently used to detect cancer or are under development. Furthermore, understanding the impact of gene mutations/variants is essential to cancer prognosis.

Although the OvC is extensively studied, its heterogenity and broad range of origin make the treatment difficult. For that reason, cancer therapy tailored to the particular genetic background of the disease offers new hope. However, further research is still needed to be knowledgeable in all physiological aspects and health hazards of the disease. It will particularly be dedicated to:

1) Proteomics and regulation of candidate DNA repair genes with function in OvC
2) Functional aspects of DNA repair system in OvC
3) Mechanistic studies of DNA repair function in OvC cells.
5 References


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