DNA Repair Mechanisms in Colorectal Carcinogenesis

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Abstract: Colon cancer is among the most common cancers and the third cause of cancer deaths worldwide. If detected at an early stage, treatment might often lead to cure. The present review adduces the so far studied alterations in the expression of genes, as well as polymorphisms of genes engaged in DNA repair systems, with particular emphasis on indirect ones that are correlated with colorectal cancer. Such aberrations could be linked to an increased risk for the development of colorectal cancer and might serve as potential targets in the areas of prevention and therapy.

Keywords: Biomarker, colorectal carcinogenesis, DNA repair, gene polymorphism, genetic instability, genetic predisposition.

1. INTRODUCTION

Colorectal cancer (CRC) is among the most common cancers worldwide and the third cause of cancer mortality [1]. Two different genetic pathways are involved in the evolution of normal mucosa to adenoma and carcinoma. The first is subjected to accumulation of mutations (chromosomal instability, CIN) in oncogenes and tumor-suppressor genes like K-*Ras*, *TP53* and *APC* [2, 3]. APC germline mutations are correlated with the familial adenomatous polyposis (FAP) syndrome, a heritable type of colon cancer. The second pathway incorporates alterations in mismatch repair (MMR) genes [3, 4]. In this pathway the dominant syndrome is hereditary non-polyposis colon cancer (HNPCC), which is correlated with germline mutations. On the other hand, if the mutations are observed in somatic cells, the tumors are depicted as microsatellite instability (MSI) ones, a type that is more frequent and also responsible for sporadic tumors of the colon [4-7]. All the aforementioned pathways are characterized by impairment of DNA repair systems that predisposes cells to genetic instability, which is considered a hallmark of cancer.

This review describes the malfunction of the DNA repair systems and discusses its relation to the risk of developing colorectal carcinoma.

2. DNA REPAIR MECHANISMS

DNA alterations can be imported either by the DNA replication system, mainly due to the replication slippage of DNA polymerase or due to insertion of false bases leading to mismatches. Environmental and intracellular factors can also induce modifications in sporadic DNA bases [8]. Depending on the specific type of alteration in DNA double helix and the phase of the cell cycle, different repair mechanisms are potentiated to restore the damage. In most of the cases, cells use the unmodified complementary strand of the DNA or the sister chromatid as a template to restore the original strand. Without access to a template, cells use an error-prone recovery mechanism known as translesion synthesis as a last resort.

Repair mechanisms are divided into direct and indirect ones. The first incorporate repair during replication, catalyzed by the main DNA polymerase, the methylguanine-DNA methyltransferase (MGMT) enzyme repair for damages in O6 position of guanine caused by endogenous and methylating (alkylating) agents and finally the repair of pyrimidine dimers in bacteria and plants catalyzed by photolyase [9-11]. Indirect repair mechanisms are divided into three categories: excision repair (ER), recombination repair (RR) and MMR. ER is further divided into two subcategories: base excision repair (BER) for abnormal bases such as uracil and breaks found only in one DNA strand and nucleotide excision repair (NER) for the removal of bulky adducts. In BER, DNA glycosylase is activated in alterations of DNA bases due to damages induced by intracellular factors and XRCC1 in removal of bases due to radiation breakage. APE1 recognizes the abasic site and hydrolyzes the phosphodiester bond. The repair is then catalyzed by DNA polymerase β and DNA is joined by DNA ligase [12-16]. In NER, XPC enzyme spots the damage and then DNA is unfolding from both sides in the position of damage due to the presence of helicases XPA, XPG and TFIIH. XPF and XPG enzymes are responsible for the double excision in 5′ and 3′ direction, respectively, around the damage. At a last step, the removal of the DNA follows the synthesis of a new strand [17]. RR corrects DNA double-strand breaks (DSBs) as well as interstrand crosslinks and involves two subpathways: homologous recombination (HR) that dominates during S and G2 phase of the cell cycle, and non-homologous end joining (NHEJ) which is active in G1 phase. Ataxia Telangiectasia Mutated (ATM) kinase exerts an important role in both pathways [18-21]. MMR recognizes and repairs small loops in DNA either by base–base mismatches or by insertion/deletion loops that arise from nucleotide misincorporation [22-29].

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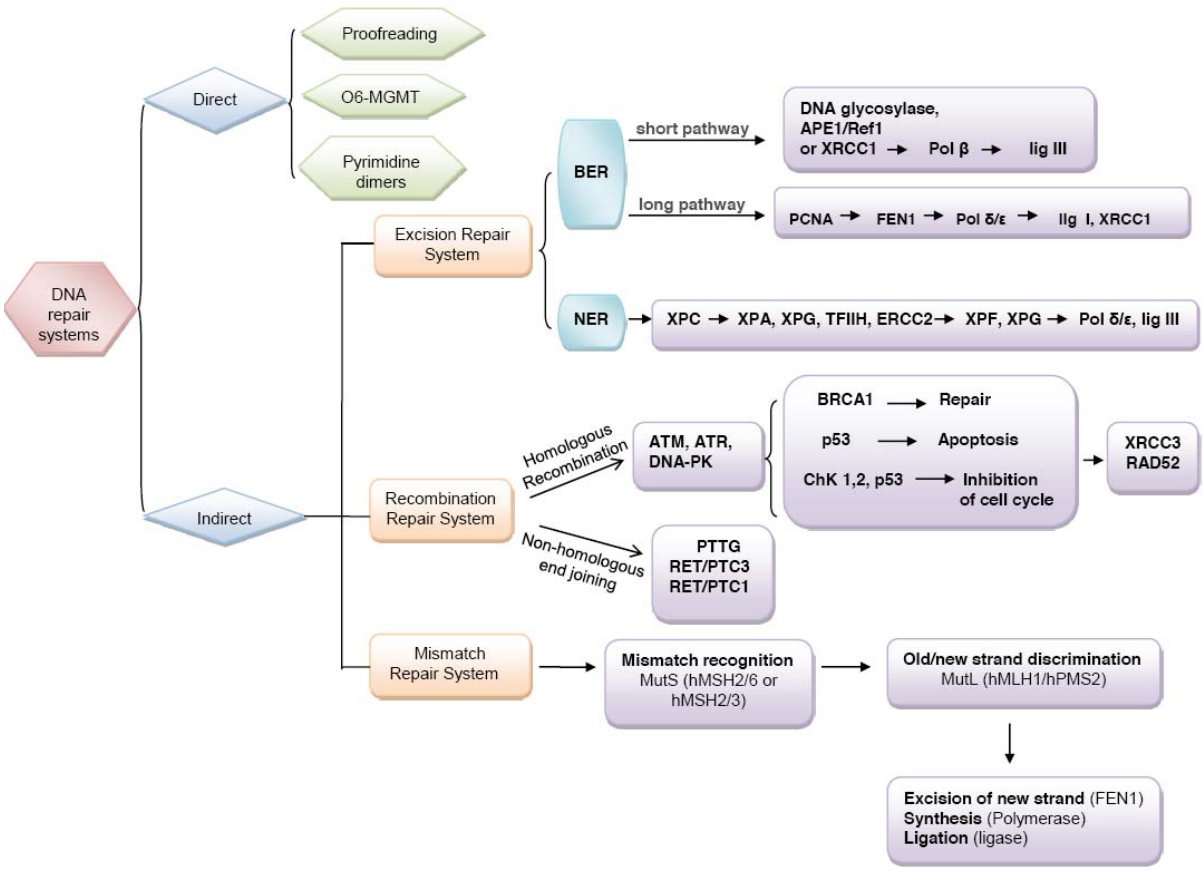

Fig. (1). Schematic overview of the DNA repair mechanisms (for abbreviations see text). Adapted and reprinted in modified form from Ref [29], with permission © 2010 Elsevier.

False base pairment or loops are recognized by hMSH2 protein that forms a heterodimer with either hMSH6 for classical mismatches and single-base loops or hMSH3 for bigger loops. The heterodimer MLH1–PMS2 discriminates the old from the new strand and then FEN1 excises the new strand, followed by DNA polymerase and ligase. All the above mechanisms are summarized in Fig. (**1**).

3. DNA REPAIR SYSTEMS IN COLORECTAL CARCINOGENESIS (FIG. (2))

3.1. NER and Colon Cancer

DNA excision repair protein ERCC-1 holds a predominant role in the excision mechanism. The expression of this protein is frequently reduced or absent in CRC patients [30]. These aberrant expression levels create cells with augmented ability to survive due to blockade of apoptosis and increased mutability, suggesting that ERCC-1 abnormal expression may comprise an early step in colon cancer progression.

Xeroderma pigmentosum group D (*XPD*) gene product bears an important role in NER pathway being responsible for opening DNA around the damaged site, a crucial step for the initiation of the NER mechanism that repairs bulky adducts and UV-induced DNA damage [31-33]. The XPD protein has ATP-dependent helicase activity and is linked to the initiation transcription factor IIH (TFIIH) [32]. Two *XPD* polymorphisms, Asp312Asn in exon 10 and Lys751Gln in exon 23, have been identified [34]. These genotypes are associated with a lower efficacy of damage repairing induced by UV and chemical carcinogens [35, 36]. Moreover, it has been found that variation in the *XPD* Lys751Gln gene may alter the XPD protein's function and modifies DNA repair capacity depending on different exposures, while it seems to favor higher proficiency in repairing the damage induced by ionizing radiation [35, 37]. Several studies have shown a minor correlation of *XPD* Lys751Gln polymorphism with CRC risk [38-42]. The 312Asn allele has no reported effect on DNA repair capacity [43].

3.2. BER and Colon Cancer

Apurinic/apyrimidinic endonuclease 1 (APE1) is a bifunctional AP endonuclease/redox factor involved in DNA repair and redox signaling, and requires Mg^{2+} in its active site to actively participate in BER mechanism. APE1 has also been linked to radioresistance. Specifically, it was examined whether targeted inhibition of APE1 can sensitize tumor cells to irradiation *in vitro* and *in vivo*. Chimeric adenoviral vector Ad5/F35 carrying human APE1 small interfering

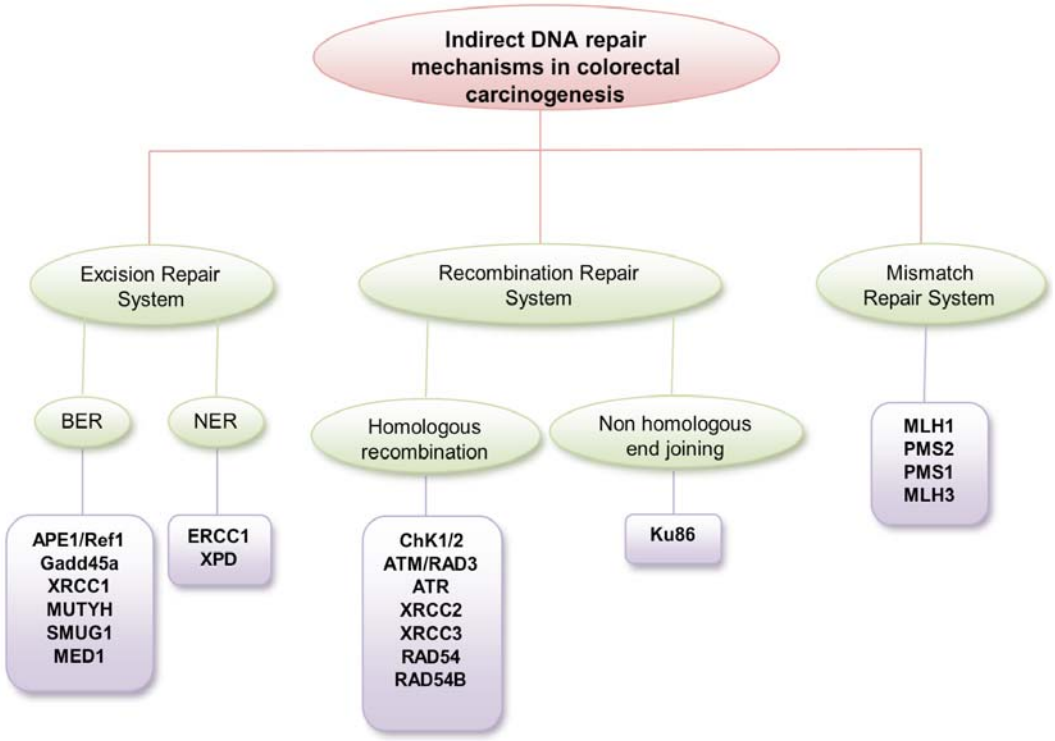

Fig. (2). DNA repair genes involved in colorectal carcinogenesis (for abbreviations see text).

(si) RNA (Ad5/F35-APE1 siRNA) was constructed and its infectivity was found to be greater than that of Ad5 alone in LOVO colon cancer cells. The expression of APE1 was as strong as that of nuclear factor-κB (NF-κB), the downstream molecule of APE1, known for its radioresistance activity. Ad5/F35-APE1 siRNA significantly enhanced sensitivity of LOVO cells to irradiation in clonogenic survival assays, and impairment of tumor growth by irradiation was demonstrated in a nude mouse colon cancer model once inhibiting the expression of APE1 protein in LOVO xenografts [44].

Growth arrest and DNA damage inducible protein alpha (*Gadd45a*) is a p53-regulated gene that has been shown to delay carcinogenesis and decrease mutation frequency. Its expression levels are elevated after stressful growth arrest conditions and treatment with DNA-damaging agents [45]. The gene is known to regulate NER mechanism in response to UV radiation, while an emerging role in BER mechanism has also been reported [46]. *Gadd45a*-null mouse embryo fibroblasts MEF and *gadd45a*-deficient human colon cancer cells exhibited slow BER after treatment with methyl methanesulfonate, a pure base-damaging agent. Moreover, the removal of AP sites by APE1/redox factor 1 (APE1/Ref1) was significantly delayed in *gadd45a*-null cells. Furthermore, APE1/Ref1 was localized in the nucleus of *gadd45a* wild-type cells, whereas in *gadd45a*-deficient cells APE1 was distributed in the cytoplasm exhibiting a reduced interaction with proliferating cell nuclear antigen (PCNA). Therefore, *gadd45a* seems to play a crucial role as a component gene of the p53 pathway, being involved in protection from carcinogenic base damage and maintenance of genomic stability.

DNA repair gene *X-ray repair cross-complementing groups 1* (*XRCC1*) is involved in the efficient repair of DNA strand breaks formed by exposure to ionizing radiation and alkylating agents. The gene codes for a scaffolding protein that interacts with DNA polymerase beta, DNA ligase, polynucleotide kinase, poly(ADP-ribose) polymerase and human AP endonuclease [47-50]. The distributions of the single-nucleotide polymorphism (SNP) *XRCC1* Arg399Gln and the associations of this genetic polymorphism, along with polymorphisms in other genes engaged in different DNA repair mechanisms, with CRC susceptibility have been detected, as well as the gene–gene and gene–environment interactions [38]. The *XRCC1* 399Gln allele has been found to be correlated with a significantly increased rectal cancer risk among men.

The human MutY homolog (MUTYH) functions as a DNA glycosylase responsible for excision of adenines misincorporated opposite 8-oxo-7,8-dihydro-2´ deoxyguanosine (8-oxoG), a stable product of oxidative DNA damage [51]. Cells defective in MUTYH display a mutator phenotype [52-54]. Inherited biallelic mutations in the human *MUTYH* gene have been implicated in adenomatous colorectal polyposis (MUTYH associated polyposis, MAP) and thus in an increased risk of CRC [55-61]. Mutations in both alleles of human *MUTYH* correlate with an increase in G:C to T:A transversions in the somatic *APC* gene in tumors of MAP patients, suggesting that MUTYH-dependent repair is defective [55, 57]. The Tyr165Cys, 1103delC and Gly382Asp

gene mutations have been studied in cells [62]. Tyr165Cys is part of one of the two helix–hairpin–helix motifs, which is thought to be crucial for recognition and binding of the mispaired substrates and is evolutionarily highly conserved among MUTYH homologs [63]. MUTYH Tyr165Cys is homozygous for an A to G transition at position 494 in both *MUTYH* alleles, MUTYH Gly382Asp is homozygous for a G to A transition at position 1145 in both *MUTYH* alleles and MUTYH 1103delC/Gly382Asp is compound heterozygous with a G to A transition at position 1145 in one allele and a deleted cytosine nucleotide at position 1103 in the other. The Tyr165Cys and 1103delC mutations significantly reduce MUTYH protein stability hence repair activity, whereas the Gly382Asp mutation produces a dysfunctional protein only suggesting different molecular mechanisms by which the MAP phenotype may contribute to the development of CRC.

Mediator of RNA polymerase II transcription subunit 1 (MED1) is an enzyme encoded by *MED1* gene. MED1 is a BER enzyme that interacts with MLH1 protein which is involved in MMR and maintains genomic integrity by binding methylated DNA and repairing spontaneous deamination events [64, 65]. *MED1* mutations have been associated with high MSI tumors and enhanced colorectal tumorigenesis. Promoter methylation of *MED1* gene might constitute an alternative, epigenetic mechanism for gene suppression during sporadic colorectal tumorigenesis [66]. *MED1* is also methylated and its expression is suppressed in normal-appearing colonic mucosa prior to the development of the adenoma–carcinoma sequence. The epigenetic alteration of the tumor-suppressor gene *MED1* seems to be associated with tumor initiation rather than tumor progression. This is confirmed by the lack of a significant decrease in *MED1* expression correlated with more advanced tumor stages, a condition that would imply a role in tumor progression.

3.3. HR and Colon Cancer

Checkpoint kinases 1 and 2 (Chk1 and Chk2) are structurally unrelated but have a similar function as Ser/Thr kinases possessing a crucial role in cell-cycle control [67, 68]. Chk1 for entry into mitosis and Chk2 as a cell-cycle checkpoint regulator and putative tumor suppressor, stabilize p53 leading to cell-cycle arrest in G1 [69]. Both kinases are known to relay the checkpoint signals from the upstream signal transducing kinases of the phosphatidylinositol kinase-related family, particularly the ATM and ataxia telangiectasia and RAD3 (ATR) related, to the proximal substrates [70]. Chk1, which is activated by phosphorylation at Ser345 or Ser317, phosphorylates in turn Cdc25A/C ultimately arresting cells in late S or G2 phases, whereas activation of Chk2 in checkpoint signaling is initiated by phosphorylation at Thr68 in an ATM-dependent manner [71-73]. Recent studies have attributed some extra roles to Chks, such as DNA repair control, genomic stability and apoptosis [74-76]. Phosphorylation-mediated potentiation of Chk1 is mainly dependent on ATR, whereas Chk2 is activated by ATM. Camptotechin (CPT) has been used to inhibit topoisomerase I and to explore DNA damage response of Chk1 and Chk2 in human colon cancer HCT116 cells. Degradation of Chk1 and abolishment of its phosphorylated (p) form was observed, while phosphorylation of Chk2 was increased [77]. These findings led to the conclusion that Chk1 is preferably involved in CPT-stimulated HR repair, whereas Chk2 plays a predominant role in inducing G2–M-phase arrest as well as in protecting cells from apoptosis following CPT treatment. Another study reported that defects in these two Ser/Thr kinases might contribute to the development of both hereditary and sporadic human cancers [78], since expression of Chk2 and pChk2 was decreased in ~50% of the studied cases. Quantitative studies of pChk2 revealed significant decrease of pChk2 in early stages of colorectal carcinomas. Moreover, tumor invasion to local lymph nodes was associated with an increase of the pChk2 pool. These findings point to a controversial role of Chk2, either as an inactivation factor in the very first steps of carcinogenesis but also as a progressive factor in tumor invasiveness.

XRCC3 protein (encoding gene located on chromosome 14q32.3) is involved in the HR mechanism and is a member of the family of Rad51-related proteins which are necessary for the efficient repair of DNA strand breaks and DNA cross-links, as well as for correct chromosome division [79, 80]. XRCC3 directly interacts with HsRad51 and *XRCC3*-deficient cells cannot form Rad51 foci after radiation-induced damages, and also exhibit genetic instability and increased sensitivity to DNA-damaging agents [81, 82]. XRCC3 plays a key role in maintaining the genome integrity. Substitution of Thr to Met in codon 241 (Thr241Met) due to the C18067T transition, is the most frequent polymorphism in *XRCC3* that may affect the enzyme's function and alter DNA repair capacity as well [83, 84]. It has also been observed that the *XRCC3* 241Met allele and family history of cancer might contribute to colorectal carcinogenesis, whereas no correlation between *XRCC3* polymorphism and alcohol drinking or cigarette smoking has been noticed. *XRCC3* 241Met allele has been also shown a protective tendency against rectal cancer for both men and women [38].

RAD54 is one of the key proteins necessary for HR and DNA repair. RAD54 was initially described in the budding yeast *Saccharomyces cerevisiae* as a member of the evolutionarily conserved RAD52 epistasis group which is believed to participate in DNA recombination and repair mechanisms, especially those involving double-strand breaks during both mitosis and meiosis [85]. RAD54-deficient DT40 cells are extremely sensitive to ionizing radiation in G2 as well as G1 phase, while wild-type DT40 cells are resistant to irradiation in G2 [86]. The main template for recombination in G2 phase are the sister chromatids.

Therefore, hypersensitivity to irradiation of the RAD54 mutant in G2 suggests that RAD54 is required for DNA repair mediated by sister chromatids.

These phenotypes of RAD54 mutants have not been confirmed in human RAD54B-deficient cells, supporting the notion that RAD54 acts in HR in a different manner to RAD54B. RAD54B is a protein which belongs to DEAD-like helicase superfamily [87]. It binds to double-stranded DNA, and displays ATPase activity in the presence of DNA [88]. It has been proposed that RAD54B plays a critical role in targeted integration in human cells, as experiments in a colon cancer cell line where RAD54B was inactivated resulted in severe reduction of targeted integration frequency. On the other hand, cell growth and cell survival to DNA-damaging agents or sister chromatids exchange were not affected. Given the fact that RAD54B shares structural similarity with *Saccharomyces cerevisiae* TID1/RDH54 not only in ATPase domains but also in the N-terminal region, it is possible that RAD54B is a human homolog of TID1/RDH54. In yeast, the complex TID1/RDH54 acts in the RR pathway through roles partially overlapping with those of RAD54 [86, 87].

DNA repair protein XRCC2 is a member of the RecA/Rad51-related protein family that participates in HR to maintain chromosome stability and repair DNA damage [89]. The results of a recent meta-analysis highlight the significant association between *XRCC2* intron 2 variant (rs3218499 G→C) and increased risk of rectal cancer [90].

3.4. NHEJ and Colon Cancer

Little is known about genes that are implicated in NHEJ in colon cancer. The product of DNA repair gene *Ku86* which is active in DNA NHEJ and is required for telomere length maintenance and subtelomeric gene silencing (also interacting with cytochrome c oxidase subunit I that is involved in apoptosis), has been shown to present a decreased expression in mucosal areas close to colon cancers [30].

3.5. MMR and Colon Cancer

Among MutL homologues, MLH1 mutations are by far the most common cause of HNPCC (Lynch Syndrome), whereas PMS1, PMS2 and MLH3 mutations are rare [91-94]. A mouse model with deficiency in *MLH3*, or along with *PMS2* deficiency has been examined and it was found that *MLH3* gene contributes to mechanisms of tumor suppression. *MLH3* deficiency alone causes MSI, impaired DNA damage response and increased gastrointestinal tumor susceptibility. Moreover, *MLH3*/*PMS2* double-deficient mice have shorter life span, tumor susceptibility, MSI and DNA damage response phenotypes that are indistinguishable from *MLH1*-deficient mice [95]. Both MLH1–MLH3 and MLH1–PMS2 complexes seem to be involved in mismatch repair functions assigning tumor suppression, providing an explanation why among families with Lynch syndrome only *MLH1* mutations are the most frequently presented.

The core promoter of *MLH1* contains a common SNP (-93G→A, dbSNP ID: rs1800734) located in a region essential for maximum transcriptional activity. There are studies which associate this variant with an increased risk of developing hyperplastic colonic polyps in smokers, CRC in people with family history of the disease and presence of MSI [96-98]. In an analysis of 1518 CRC patients, homozygosity for the *MLH1* -93A variant was associated with a three-fold risk of CRC negative for MLH1 protein by immunohistochemistry. The analysis demonstrated a positive correlation between *MLH1* -93A variant and risk of developing MMR-deficient CRC, particularly with somatic loss of MLH1 protein expression [99].

MutL homolog, human PMS2, a protein active in DNA mismatch repair and necessary for apoptosis of cells with excessive DNA damage can also cause a disruption of the MMR pathway in mammalian cells, resulting in hypermutability and DNA damage tolerance once the protein is overexpressed [100]. The overexpression of either wild-type or truncated human PMS2, in otherwise wild-type mouse cells, results in both spontaneous and damage-induced hypermutability [100]. PMS2 overexpression also assigns a DNA damage tolerance phenotype similar to that observed with MMR deficiency. These data indicate that aberrant expression of one component of the MMR complex can disrupt MMR function and contribute to genetic instability, thereby increasing the risk of carcinogenesis.

MSI is a hypermutable phenotype caused by the loss of DNA MMR activity [101]. MSI occurs in 10-20% of CRCs and has been linked to *MLH1* promoter hypermethylation as well as to germline mutations in MMR genes [102]. Colorectal tumors with MSI have defined characteristics, such as a tendency to arise in the proximal colon, lymphocytic infiltrate and a poorly differentiated mucinous or signet ring appearance [101]. High MSI is a feature of Lynch syndrome, which is a rare inherited disorder caused by germline mutation in MMR genes. Tumors with high MSI phenotype seem to have a slightly better prognosis than colorectal tumors without MSI and do not exhibit the same response to chemotherapeutics [102, 103].

Clinical studies focusing on the survival benefit from 5- fluorouracil (5-FU) chemotherapy are still contradictory. Some of them report associations between MSI-H tumors and good survival benefit or better response [104]. Other studies depict no apparent survival benefit [105]. MSI-H phenotype is related with resistance to cisplatin and carboplatin but not to oxaliplatin [106, 107], whereas adjuvant chemotherapy with FOLFOX (a combination of oxaliplatin, 5-FU and leucovorin) in this tumor category concluded zero effect [108]. Irinotecan, a topoisomerase I inhibitor, seems to possess a positive effect on MSI-H tumors being related with an increase in survival [109].

A study covering population-based and clinical-based cases evaluated the molecular characteristics of high MSI CRC, in order to delineate whether all high MSI tumors can be explained by either germline mutation in one of the MMR genes or *MLH1* gene methylation. *MLH1* methylation was noted in 60% of population-based high MSI tumors and 13% of clinical-based high MSI colorectal tumors. The data reveal that *MLH1* methylation may explain high MSI CRC in the absence of a detected germline mutation in some of these cases. The low frequency of *MLH1* methylation in clinical-based cases with a germline mutation also indicates that germline mutation and methylation are independent mechanisms for inactivation of *MLH1*, and that the remaining wild-type allele in most Lynch syndrome cases is not typically inactivated by DNA methylation. As far as the population-based study is concerned, germline mutation in one of the MMR genes was higher in cases diagnosed before the age of 50 years than in cases diagnosed after the age of 50 years (39% vs 9%, respectively), while the prevalence of *MLH1* methylation was higher in cases diagnosed after the age of 50 years (63% vs 14% before the age of 50 years). These observations suggest that older age of diagnosis constitutes an independent predictor of *MLH1* methylation, and that a vast majority of high MSI CRC could be explained by either germline mutation within one of the MMR genes or *MLH1* methylation [110].

4. THERAPEUTIC RELEVANCE OF DNA REPAIR MECHANISMS IN COLON CANCER

To our knowledge, treatment of colorectal cancer patients has not been based on the efficiency of DNA repair machinery. During the last decade exists only a strict number of studies targeting abnormalities in DNA repair mechanisms examining the possible effect of chemotherapy in a subgroup of patients subjected to such malfunctions.

In our days, it is well established that cells with a deficient DNA MMR system are resistant to radiation and to many classes of cytotoxic chemotherapy. The platinum-containing drugs cisplatin and carboplatin, alkylating (busulfan) and methylating agents (procarbazine and temozolomide), the antimetabolites 6-thioguanine and 5-fluorouracil (5-FU), and the topoisomerase II inhibitors etoposide and doxorubicin are among these classes [111]. Yang and his team worked on Carboxyamidotriazole (CAI), a new generation calcium channel blocker (CCB) with antitumor activity [112, 113] and its effect in various cell lines (including colon cancer) with known MMR status, based on previous *in vitro* and *in vivo* studies depicting CAI to possess antiinvasive, antimetastatic and antiangiogenic properties [114, 115]. They concluded that CAI unlike high-dose nifedipine (another CCB) is better suited for clinical use, possessing a selective antiproliferative effect in DNA MMR deficient colon cancer cells. In addition, CAI (at 10 μM) can inhibit *in vitro* invasion by MMR-deficient cell lines, but not in their MMR-proficient counterparts. Moreover, CAI (3 μM) induced a greater degree of apoptosis and similar level of G2/M arrest in MMR (hMLH1- or hMSH6-) deficient colon cancer cells than in the matched proficient cell lines [112].

In the field of treatment with 5-FU, effects remain controversial as discussed previously in the section of the MSI-H tumors. Patients with deficient MMR colon cancers have reduced rates of tumor recurrence, delayed time to recurrence, and improved survival rates, compared with proficient MMR colon cancers. Furthermore, distant recurrences were reduced by 5-FU based adjuvant treatment in deficient MMR stage III tumors, and a subset analysis suggested that any treatment benefit was restricted to suspected germline vs sporadic tumors [116]. Clinical data suggest that patients with MMR-deficient cancers do not benefit from therapies with fluorinated pyrimidine (FP) derivatives [117]. MSH2 deficiency, due to tumors' resistance to standard treatments such as 5-FU, can be targeted by therapies that cause oxidative DNA damage, and in particular methotrexate [118].

NER, as mentioned before, is the main cellular process interfering in the removal of bulky adducts such as oxaliplatin–DNA platinum ones [119]. In this machinery, ERCC1 is the crucial enzyme leading to the 5' incision of the platinum DNA damage [120]. A combination of oxaliplatin/cetuximab found to inhibit oxaliplatin-induced overexpression of ERCC1 through transcriptional regulation in HCT-8 cells, inducing a dramatic decrease in cells in the S phase and enrichment in the G1 and G2/M phases. In addition, oxaliplatin/cetuximab combination seems to promote an early apoptotic effect and to inhibit survival through downregulation of AKT activation in HCT-8 cells. Moreover, the same study concluded that Cetuximab enhances oxaliplatin-induced downregulation of multiple targets involved in DNA replication, recombination, and repair [121].

5. CONCLUDING REMARKS

This review summarizes the so far available information concerning indirect DNA repair mechanisms (excision, recombination and mismatch repair systems) and colorectal carcinogenesis. Alterations in specific genes have been characterized and appear to be related with tumor formation, progression and metastasis. Alterations have also been confirmed in normal mucosa and appear to serve as the initiative step in carcinogenesis. A variety of polymorphisms have been found to display different response to radiation or antitumor drugs. Discovery of specific alterations in colorectal tumors have increased awareness of the diversity of CRCs. We are now in a stage to search for possible biomarkers among these altered genes and among the genes they interact with through the different pathways that cross-talk.

These perspectives may alter the general WHO guidelines to more specific ones according to each

person's genetic profile, and adjuvant chemotherapy will be considered as a plus in cases that was not used before, e.g. patients in stage II who may not receive an extra therapy after surgery. Thus, the more prone to experience the disease again will take an advantage, while those without genetic alterations will be still away of any extra treatment. Given the fact that chemotherapy is accompanied by various side effects, it will be wise to divide patients in groups and administer therapy only to those with a greater possibility to face cancer again.

On the other hand, it seems feasible to search for biomarkers in easy accessible material, like blood and other body fluids without any interventional method. Individuals will be more willing to be tested as the sampling will not cause injury, pain or deterioration of the material like other methods. Tests could be part of a routine examination as stands nowadays with PSA for prostate, covering all the carcinogenetic steps from alterations in normal mucosa that encrypt susceptibility, to deterioration to adenoma and progression to cancer and metastasis. DNA methylation patterns may also be prognostic factors of metastatic or aggressive CRC. Tumors will demonstrate a better response to chemotherapy, as according to the damage, they will be handled separately. Circulating tumor cells, another emerging field in the area of prognosis, may serve as well in this vein. The persistent presence of tumor cells in peripheral blood of the patients receiving chemotherapy may evince the inadequacy or the unsuitability of the followed therapy and alternative remedies will be examined in order to benefit the patient. Personalized treatment could serve as a powerful tool against cancer, as not only CRCs but also the majority of cancer types are characterized by diversity. Targeted and individually therapy raises as "the bump in the root of the problem".

CONFLICT OF INTEREST STATEMENT

All authors state no financial and personal relationships with other people or organizations that could inappropriately influence this work.

ACKNOWLEDGEMENTS

C. Michailidi is supported by a grant from 'The Stavros Niarchos Foundation'.

REFERENCES

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61: 759-67.

[3] Gervaz P, Bouzourene H, Cerottini JP, *et al*. Dukes B colorectal cancer: distinct genetic categories and clinical outcome based on proximal or distal tumor location. Dis Colon Rectum 2001; 44: 364-72.

[4] Miyakura Y, Sugano K, Konishi F, *et al*. Extensive methylation of hMLH1 promoter region predominates in proximal colon cancer with microsatellite instability. Gastroenterology 2001; 121: 1300-9.

[5] Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. Science 1993; 260: 816-9.

[6] Delattre O, Olschwang S, Law DJ, *et al*. Multiple genetic alterations in distal and proximal colorectal cancer. Lancet 1989; 334: 353-6.

[7] Patchett SE, Alstead EM, Saunders BP, Hodgson SV, Farthing MJ. Regional proliferative patterns in the colon of patients at risk for hereditary nonpolyposis colorectal cancer. Dis Colon Rectum 1997; 40: 168-71.

[8] Friedberg EC, McDaniel LD, Schultz RA. The role of endogenous and exogenous DNA damage and mutagenesis. Curr Opin Genet Dev 2004; 14: 5-10.

[9] Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature 2001; 411: 366-74.

[10] Pecorino L. Molecular biology of cancer: Mechanisms, targets, and therapeutics, 1rst ed. New York: Oxford University Press 2005.

[11] Sancar A. Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. Chem Rev 2003; 103: 2203-37.

[12] Lindahl T. Suppression of spontaneous mutagenesis in human cells by DNA base excision-repair. Mutat Res 2000; 462: 129-35.

[13] Nilsen H, Krokan HE. Base excision repair in a network of defense and tolerance. Carcinogenesis 2001; 22: 987-98.

[14] Krokan HE, Nilsen H, Skorpen F, Otterlei M, Slupphaug G. Base excision repair of DNA in mammalian cells. FEBS Lett 2000; 476: 73-7.

[15] Memisoglu A, Samson L. Base excision repair in yeast and mammals. Mutat Res 2000; 451: 39-51.

[16] Frosina G, Fortini P, Rossi O, *et al*. Two pathways for base excision repair pathways in mammalian cells. J Biol Chem 1996; 271: 9573-8.

[17] Friedberg EC. How nucleotide excision repair protects against cancer. Nat Rev Cancer 2001; 1: 22-33.

[18] Paques F, Haber JE. Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 1999; 63: 349-404.

[19] Jackson SP. Sensing and repairing DNA double-strand breaks. Carcinogenesis 2002; 23: 687-96.

[20] Thompson LH, Schild D. Recombinational DNA repair and human disease. Mutat Res 2002; 509: 49-78.

[21] Collis SJ, DeWeese TL, Jeggo PA, Parker AR. The life and death of DNA-PK. Oncogene 2005; 24: 949-61.

[22] Evans E, Alani E. Roles for mismatch repair factors in regulating genetic recombination. Mol Cel Biol 2000; 20: 7839-44.

[23] Kolodner RD, Marsischky GT. Eukaryotic DNA mismatch repair. Curr Opin Genet Dev 1999; 9: 89-96.

[24] Buermeyer AB, Deschenes SM, Baker SM, Liskay RM. Mammalian DNA mismatch repair. Annu Rev Genet 1999; 33: 533-64.

[25] Marti TM, Kunz C, Fleck O. DNA mismatch repair and mutation avoidance pathways. J Cell Physiol 2002; 191: 28-41.

[26] Harfe BD, Jinks-Roberton S. DNA mismatch repair and genetic instability. Annu Rev Genet 2000; 34: 359-99.

[27] Hsieh P. Molecular mechanisms of DNA mismatch repair. Mutat Res 2001; 486: 71-87.

[28] Acharya S, Wilson T, Gradia S, *et al*. hMSH2 forms specific mispair-binding complexes with hMSH3 and hMSH6. Proc Natl Acad Sci USA 1996; 93: 13629-34.

[29] Gatzidou E, Michailidi C, Tseleni-Balafouta S, Theocharis S. An epitome on DNA repair related genes and mechanisms in thyroid carcinoma. Cancer Lett 2010; 290: 139-47.

[30] Nguyen H, Loustaunau C, Facista A, *et al*. Deficient Pms2, ERCC1, Ku86, CcOI in field defects during progression to colon cancer. J Vis Exp 2010; 41. pii: 1931.

[31] Stern MC, Butler LM, Corral R, *et al*. Polyunsaturated fatty acids, DNA repair single nucleotide polymorphisms and colorectal cancer in the Singapore Chinese Health Study. J Nutrigenet Nutrigenomics 2009; 2: 273-9.

[32] Egly JM. The 14th Datta Lecture TFIIH: from transcription to clinic. FEBS Lett 2001; 498: 124-8.

[33] Weeda G, Hoeijmakers JH. Genetic analysis of nucleotide excision repair in mammalian cells. Semin Cancer Biol 1993; 4: 105-17.
[34] Shen MR, Jones IM, Mohrenweiser H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res 1998; 58: 604-8.
[35] Spitz MR, Wu X, Wang Y, *et al*. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. Cancer Res 2001; 61: 1354-7.
[36] Qiao Y, Spitz MR, Guo Z, *et al*. Rapid assessment of repair of ultraviolet DNA damage with a modified host-cell reactivation assay using a luciferase reporter gene and correlation with polymorphisms of DNA repair genes in normal human lymphocytes. Mutat Res 2002; 509: 165-74.
[37] Moller P, Knudsen LE, Frentz G, Dybdahl M, Wallin H, Nexo BA. Seasonal variation of DNA damage and repair in patients with non-melanoma skin cancer and referents with and without psoriasis. Mutat Res 1998; 407: 25-34.
[38] Wang J, Zhao Y, Jiang J, *et al*. Polymorphisms in DNA repair genes *XRCC1*, *XRCC3* and *XPD*, and colorectal cancer risk: a case-control study in an Indian population. J Cancer Res Clin Oncol 2010; 136: 1517-25.
[39] Mort R, Mo L, McEwan C, Melton DW. Lack of involvement of nucleotide excision repair gene polymorphisms in colorectal cancer. Br J Cancer 2003; 89: 333-7.
[40] Skjelbred CF, Saebo M, Wallin H, *et al*. Polymorphisms of the XRCC1, XRCC3 and XPD genes and risk of colorectal adenoma and carcinoma, in a Norwegian cohort: a case control study. BMC Cancer 2006; 6: 67.
[41] Yeh CC, Hsieh LL, Tang R, Chang-Chieh CR, Sung FC. MS-920: DNA repair gene polymorphisms, diet and colorectal cancer risk in Taiwan. Cancer Lett 2005; 224: 279-88.
[42] Stern MC, Conti DV, Siegmund KD, *et al*. DNA repair single-nucleotide polymorphisms in colorectal cancer and their role as modifiers of the effect of cigarette smoking and alcohol in the Singapore Chinese Health Study. Cancer Epidemiol Biomarkers Prev 2007; 16: 2363-72.
[43] Lunn RM, Helzlsouer KJ, Parshad R, *et al*. XPD polymorphisms: effects on DNA repair proficiency. Carcinogenesis 2000; 21: 551-5.
[44] Xiang DB, Chen ZT, Wang D, *et al*. Chimeric adenoviral vector Ad5/F35-mediated APE1 siRNA enhances sensitivity of human colorectal cancer cells to radiotherapy *in vitro* and *in vivo*. Cancer Gene Ther 2008; 15: 625-35.
[45] Hollander MC, Alamo I, Jackman J, Wang MG, McBride OW, Fornace AJ. Analysis of the mammalian gadd45 gene and its response to DNA damage. J Biol Chem 1993; 268: 24385-93.
[46] Jung HJ, Kim EH, Mun JY, *et al*. Base excision DNA repair defect in Gadd45a-deficient cells. Oncogene 2007; 26: 7517-25.
[47] Caldecott KW, McKeown CK, Tucker JD, Ljungquist S, Thompson LH. An interaction between the mammalian DNA repair protein XRCC1 and DNA ligase III. Mol Cell Biol 1994; 14: 68-76.
[48] Gryk MR, Marintchev A, Maciejewski MW, Robertson A, Wilson SH, Mullen GP. Mapping of the interaction interface of DNA polymerase beta with XRCC1. Structure 2002; 10: 1709-20.
[49] Whitehouse CJ, Taylor RM, Thistlethwaite A, *et al*. XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair. Cell 2001; 104: 107-17.
[50] Vidal AE, Boiteux S, Hickson ID, Radicella JP. XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein–protein interactions. EMBO J 2001; 20: 6530-9.
[51] Slupska MM, Luther WM, Chiang JH, Yang H, Miller JH. Functional expression of hMYH, a human homolog of the *Escherichia coli* MutY protein. J Bacteriol 1999; 181: 6210-3.
[52] Ohtsubo T, Nishioka K, Imaiso Y, *et al*. Identification of human MutY homolog (hMYH) as a repair enzyme for 2-hydroxyadenine in DNA and detection of multiple forms of hMYH located in nuclei and mitochondria. Nucleic Acids Res 2000; 28: 1355-64.
[53] Takao M, Zhang QM, Yonei S, Yasui A. Differential subcellular localization of human MutY homolog (hMYH) and the functional activity of adenine:8-oxoguanine DNA glycosylase. Nucleic Acids Res 1999; 27: 3638-44.
[54] Hirano S, Tominaga Y, Ichinoe A, *et al*. Mutator phenotype of MUTYH-null mouse embryonic stem cells. J Biol Chem 2003; 278: 38121-4.
[55] Al-Tassan N, Chmiel NH, Maynard J, *et al*. Inherited variants of MYH associated with somatic G:C→T:A mutations in colorectal tumors. Nat Genet 2002; 30: 227-32.
[56] Jones S, Emmerson P, Maynard J, *et al*. Biallelic germline mutations in MYH predispose to multiple colorectal adenoma and somatic G:C→T:A mutations. Hum Mol Genet 2002; 11: 2961-7.
[57] Sieber O M, Lipton L, Crabtree M, *et al*. Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. N Engl J Med 2003; 348: 791-9.
[58] Halford SE, Rowan AJ, Lipton L, *et al*. Germline mutations but not somatic changes at the MYH locus contribute to the pathogenesis of unselected colorectal cancers. Am J Pathol 2003; 162: 1545-8.
[59] Enholm S, Hienonen T, Suomalainen A, *et al*. Proportion and phenotype of MYH-associated colorectal neoplasia in a population-based series of Finnish colorectal cancer patients. Am J Pathol 2003; 163: 827-32.
[60] Lipton L, Halford S E, Johnson V, *et al*. Carcinogenesis in MYH-associated polyposis follows a distinct genetic pathway. Cancer Res 2003; 63: 7595-9.
[61] Fleischmann C, Peto J, Cheadle J, Shah B, Sampson J, Houlston RS. Comprehensive analysis of the contribution of germline MYH variation to early-onset colorectal cancer. Int J Cancer 2004; 109: 554-8.
[62] Parker AR, Sieber OM, Shi C, *et al*. Cells with pathogenic biallelic mutations in the human MUTYH gene are defective in DNA damage binding and repair. Carcinogenesis 2005; 26: 2010-8.
[63] Guan Y, Manuel RC, Arvai AS, *et al*. MutY catalytic core, mutant and bound adenine structures define specificity for DNA repair enzyme superfamily. Nat Struct Biol 1998; 5: 1058-64.
[64] Hendrich B, Hardeland U, Ng HH, Jiricny J, Bird A. The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. Nature 1999; 401: 301-4.
[65] Bellacosa A, Cicchillitti L, Schepis F, *et al*. MED1, a novel human methyl CpG-binding endonuclease, interacts with DNA mismatch repair protein MLH1. Proc Natl Acad Sci USA 1999; 96: 3969-74.
[66] Howard JH, Frolov A, Tzeng CDW, *et al*. Epigenetic downregulation of the DNA repair gene *MED1/MBD4* in colorectal and ovarian cancer. Cancer Biol Ther 2009; 8: 1-7.
[67] Shao RG, Cao CX, Shimizu T, O'Connor PM, Kohn KW, Pommier Y. Abrogation of an S-phase checkpoint and potentiation of camptothecin cytotoxicity by 7-hydroxystaurosporine (UCN-01) in human cancer cell lines, possibly influenced by p53 function. Cancer Res 1997; 57: 4029-35.
[68] Yu Q, Rose JH, Zhang H, Pommier Y. Antisense inhibition of Chk2/ hCds1 expression attenuates DNA damage-induced S and G2 checkpoints and enhances apoptotic activity in HEK-293 cells. FEBS Lett 2001; 505: 7-12.
[69] Chehab NH, Malikzay A, Appel M, Halazonetis TD. Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53. Genes Dev 2000; 14: 278-88.
[70] Abraham RT. Cell cycle checkpoint signaling through the ATM and ATR kinases. Genes Dev 2001; 15: 2177-96.
[71] Mailand N, Falck J, Lukas C, *et al*. Rapid destruction of human Cdc25A in response to DNA damage. Science 2000; 288: 1425-9.
[72] PengCY, Graves PR, Thoma RS, Wu Z, Shaw AS, Piwnica-Worms H. Mitotic and G2 checkpoint control: regulation of

14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. Science 1997; 277: 1501-5.

[73] Ahn JY, Schwarz JK, Piwnica-Worms H, Canman CE. Threonine 68 phosphorylation by ataxia telangiectasia mutated is required for efficient activation of Chk2 in response to ionizingradiation. Cancer Res 2000; 60: 5934-6.

[74] Bartek J, Lukas J. Chk1 and Chk2 kinases in checkpoint control and cancer. Cancer Cell 2003; 3: 421-9.

[75] Ghosh JC, Dohi T, Raskett CM, Kowalik TF, Altieri DC. Activated checkpoint kinase 2 provides a survival signal for tumor cells. Cancer Res 2006; 66: 11576-9.

[76] Zhuang J, Zhang J, Willers H, *et al*. Checkpoint kinase 2-mediated phosphorylation of BRCA1 regulates the fidelity of nonhomologous endjoining. Cancer Res 2006; 66: 1401-8.

[77] Huang M, Miao ZH, Zhu H, Cai YJ, Lu W, Ding J. Chk1 and Chk2 are differentially involved in homologous recombination repair and cell cycle arrest in response to DNA double-strand breaks induced by camptothecins. Mol Cancer Therap 2008; 7: 1440-9.

[78] Stawinska M, Cygankiewicz A, Trzcinski R, Mik M, Dziki A, Krajewska WM. Alterations of Chk1 and Chk2 expression in colon cancer. Int J Colorect Dis 2008; 23: 1243-9.

[79] Brenneman MA, Weiss AE, NickoloV JA, Chen DJ. XRCC3 is required for efficient repair of chromosome breaks by homologous recombination. Mutat Res 2000; 459: 89-97.

[80] Stern MC, Umbach DM, Lunn RM, Taylor JA. DNA repair gene XRCC3 codon 241 polymorphism, its interaction with smoking and XRCC1 polymorphisms, and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 2002; 11: 939-43.

[81] Pierce AJ, Johnson RD, Thompson LH, Jasin M. XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. Genes Dev 1999; 13: 2633-8.

[82] Griffin CS. Aneuploidy, centrosome activity and chromosome instability in cells deficient in homologous recombination repair. Mutat Res 2002; 504: 149-55.

[83] Matullo G, Palli D, Peluso M, *et al*. XRCC1, XRCC3, XPD gene polymorphisms, smoking and (32) P-DNA adducts in a sample of healthy subjects. Carcinogenesis 2001b; 22: 1437-45.

[84] Jin MJ, Chena K, Songa L, *et al*. The association of the DNA repair gene XRCC3 Thr241Met polymorphism with susceptibility to colorectal cancer in a Chinese population. Cancer Gen Cytogen 2005; 163: 38-43.

[85] Kanaar R, Troelstra C, Swagemakers SM, *et al*. Human and mouse homologs of the *Saccharomyces cerevisiae* RAD54 DNA repair gene: evidence for functional conservation. Curr Biol 1997; 6: 828-38.

[86] Takata M, Sasaki MS, Sonoda E, *et al*. Homologous recombination and nonhomologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. EMBO J 1998; 17: 5497-508.

[87] Miyagawa K, Tsuruga T, Kinomura A, *et al*. A role for RAD54B in homologous recombination in human cells. EMBO J 2002; 21: 175-80.

[88] Hiramoto T, Nakanishi T, Sumiyoshi T, *et al*. Mutations of a novel human RAD54 homologue, RAD54B, in primary cancer. Oncogene 1999; 18: 3422-6.

[89] Cartwright R, Tambini CE, Simpson PJ, Thacker J. The XRCC2 DNA repair gene from human and mouse encodes a novel member of the recA/RAD51 family. Nucleic Acids Res 1998; 26: 3084-9.

[90] Curtin K, Lin WY, George R, *et al*. Genetic variants in XRCC2: new insights into colorectal cancer tumorigenesis. Cancer Epidemiol Biomarkers Prev 2009; 18: 2476-84.

[91] Edelmann L, Edelmann W. Loss of DNA mismatch repair function and cancer predisposition in the mouse: animal models for human hereditary nonpolyposis colorectal cancer. Am J Med Genet 2004; 129C: 91-9.

[92] Liu HX, Zhou XL, Liu T, *et al*. The role of hMLH3 in familial colorectal cancer. Cancer Res 2003; 63: 1894-9.

[93] Chapelle A. Genetic predisposition to colorectal cancer. Nat Rev Cancer 2004; 4: 769-80.

[94] Liu B, Parsons R, Papadopoulos N, *et al*. Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. Nat Med 1996; 2: 169-74.

[95] Chen PC, Dudley S, Hagen W, *et al*. Contributions by MutL homologues *Mlh3* and *Pms2* to DNA mismatch repair and tumor suppression in the mouse. Cancer Res 2005; 65: 8662-70.

[96] Iyer RR, Pluciennik A, Burdett V, Modrich PL. DNA mismatch repair: functions and mechanisms. Chem Rev 2006; 106: 302-23.

[97] Yu JH, Bigler J, Whitton J, Potter JD, Ulrich CM. Mismatch repair polymorphisms and colorectal polyps: hMLH1293G>A variant modifies risk associated with smoking. Am J Gastroenterol 2006; 101: 1313-9.

[98] Raptis S, Mrkonjic M, Green RC, *et al*. MLH1293G>A promoter polymorphism and the risk of microsatellite-unstable colorectal cancer. J Natl Cancer Inst 2007; 99: 463-74.

[99] Allan JM, Shorto J, Adlard J, *et al*. MLH1 293G>A promoter polymorphism and risk of mismatch repair deficient colorectal cancer. Int J Cancer 2008; 123: 2456-9.

[100] Gibson SL, Narayanan L, Hegan DC, Buermeyer AB, Liskay RM, Glazer PM. Overexpression of the DNA mismatch repair factor, PMS2, confers hypermutability and DNA damage tolerance. Cancer Lett 2006; 244: 195-202.

[101] Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology 2010; 138: 2073-87.

[102] Iacopetta B, Grieu F, Amanuel B. Microsatellite instability in colorectal cancer. Asia Pac J Clin Oncol 2010; 6: 260-9.

[103] Vilar E, Gruber SB. Microsatellite instability in colorectal cancer-the stable evidence. Nat Rev Clin Oncol 2010; 7: 153-62.

[104] Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B. Association of tumor site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. Lancet 2000; 355: 1745-50.

[105] Ribic CM, Sargent DJ, Moore MJ, *et al*. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003; 349: 247-57.

[106] Fink D, Nebel S, Aebi S, *et al*. The role of DNA mismatch repair in platinum drug resistance. Cancer Res 1996; 56: 4881-6.

[107] Fink D, Zheng H, Nebel S, *et al*. *In vitro* and *in vivo* resistance to cisplatin in cells that have lost DNA mismatch repair. Cancer Res 1997; 57: 1841-5.

[108] Kim ST, Lee J, Park SH. Clinical impact of microsatellite instability in colon cancer following adjuvant FOLFOX therapy. Cancer Chemother Pharmacol 2010; 66: 659-67.

[109] Fallik D, Borrini F, Boige V, *et al*. Microsatellite instability is a predictive factor of the tumor response to irinotecan in patients with advanced colorectal cancer. Cancer Res 2003; 63: 5738-44.

[110] Poynter JN, Siegmund KD, Weisenberger DJ, *et al*. Molecular characterization of MSI-H colorectal cancer by *MLHI* promoter methylation, immunohistochemistry, and mismatch repair germline mutation screening. Cancer Epidemiol Biomarkers Prev 2008; 17: 3208-15.

[111] Fink D, Aebi S, Howell SB. The role of DNA mismatch repair in drug resistance. Clin Cancer Res 1998; 4: 1-6.

[112] Yang JL, Qu XJ, Yu Y, Kohn EC, Friedlander ML. Selective sensitivity to carboxyamidotriazole by human tumor cell lines with DNA mismatch repair deficiency. Int J Cancer. 2008; 123: 258-63.

[113] Kohn EC, Liotta LA. L651582: a novel antiproliferative and antimetastasis agent. J Natl Cancer Inst 1990; 82: 54-60.

[114] Kohn EC, Felder CC, Jacobs W, *et al*. Structurefunction analysis of signal and growth-inhibition by carboxyamido-triazole, CAI. Cancer Res 1994; 54: 935-42.

[115] Hupe DJ, Boltz R, Cohen CJ, *et al*. The inhibition of receptormediated and voltagedependent calcium entry by the antiproliferative L-65 1582. J Biol Chem 1991; 266: 10136-42.

[116] Sinicrope FA, Foster NR, Thibodeau SN, *et al*. DNA Mismatch Repair Status and Colon Cancer Recurrence and Survival in Clinical Trials of 5-Fluorouracil-Based Adjuvant Therapy. J Natl Cancer Inst 2011; 103: 863-75.

[117] Li LS, Morales JC, Veigl M, *et al*. DNA mismatch repair (MMR)-dependent 5-fluorouracil cytotoxicity and the potential for new therapeutic targets. Br J Pharmacol 2009; 158: 679-92.

[118] Martin SA, McCarthy A, Barber LJ, *et al*. Methotrexate induces oxidative DNA damage and is selectively lethal to tumour cells with defects in the DNA mismatch repair gene MSH2. EMBO Mol Med 2009; 1: 323-37.

[119] Chaney SG, Campbell SL, Bassett E, Wu Y. Recognition and processing of cisplatin– and oxaliplatin–DNA adducts. Crit Rev Oncol Hematol 2005; 53: 3-11.

[120] Reed E. Platinum-DNA adduct, nucleotide excision repair and platinum based anti-cancer chemotherapy. Cancer Treat Rev 1998; 24: 331-44.

[121] Balin-Gauthier D, Delord JP, Pillaire MJ, *et al*._Cetuximab potentiates oxaliplatin cytotoxic effect through a defect in NER and DNA replication initiation. Br J Cancer 2008; 98: 120-8.