WHAT'S YOUR POISON? IMPACT OF INDIVIDUAL REPAIR CAPACITY ON THE OUTCOMES OF GENOTOXIC THERAPIES IN CANCER. PART I – ROLE OF INDIVIDUAL REPAIR CAPACITY IN THE CONSTITUTION OF RISK FOR LATE-ONSET MULTIFACTORIAL DISEASE

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ABSTRACT

The capacity for repair of damage in DNA may vary between clinically healthy people and in patients with different diseases and conditions, contributing to the risk for development of late-onset multifactorial disease, eligibility for different therapies and various therapy-related complications. At present, the effects of individual variance in DNA repair capacity in human disease are best studied in cancer. The first part of this paper briefly reviews the history of the field and the currently available biomarkers of individual repair capacity associated with the risk for development of cancer and other late-onset multifactorial diseases and conditions.

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Abbreviations: DHFR: dihydrofolate reductase; EGFR: epidermal growth factor receptor; GGR: global genome repair; HER2: human epidermal growth factor receptor 2; HNPCC: hereditary non-polyposis colorectal carcinoma; MSH: MutS homologue; MLH: MutL homologue; NER: nucleotide excision repair; NHEJ: non-homologous end joining; TCR: transcription-coupled repair; TP53: tumour protein 53 (p53)

As you approach the fire it illuminates,
Warms up and finally burns.
Thoughts of Vantala (c. XII century).

Introduction

Emergence of the concept for inter-individual variance in the capacity for repair of DNA damage

The operating principle of most of the modern anticancer therapies is genotoxicity, that is, they work by inflicting massive amounts of DNA damage upon rapidly cycling cancer cells so that they are forced into growth arrest or, if possible, programmed cell death. The genotoxic properties of various chemicals and physical agents had been used in the treatment of human cancers even before the mechanisms of infliction of DNA damage, damage detection and repair and damage-induced cell death were made clear. The first genotoxic agents to be used in treatment of cancer – the nitrogen mustards – were applied in the therapy of lymphoma back in the early 1940s. This was an empirical treatment, developed after analysis of repeated reports about one of the serious adverse effects of sulfur mustards (at the time commonly used as chemical warfare agents), profound myelosuppression (34). It was more than two decades before the pioneering report of Cleaver from 1968, revealing that a rare severe inherited disease (xeroderma pigmentosum) was actually associated with deficiency in repair of DNA damage (22). This was the first paper to outline the association between defects in DNA repair and human pathology and it laid the foundations of the research in the vast and intensively developing field of mammalian DNA repair. It was quite later on, however, that the researchers eventually realised that the rate and/or the efficiency of the repair of DNA damage varied between clinically healthy people and in patients with different diseases and conditions and that the efficiency of repair of damage induced by genotoxic therapy could be an important factor for the outcomes of a treatment and the therapy-associated complications. The individual factors that may predict better or poorer response to anticancer therapy and the possible adverse effects have been of intense interest since the early days of modern oncology, as there are always patients that experience beneficial effects of a certain treatment, whereas some do not, and patients that suffer from severe therapy-related toxicity, while others have only mild symptoms. Initially, it was believed that variance in the efficiency of DNA repair would only be associated with disease phenotypes, as it was indeed demonstrated by Cleaver (22) on the example of xeroderma pigmentosum. A couple of decades later, the associations between defects in almost all genes coding for products acting in DNA repair and the corresponding disease phenotypes were made clear. The idea of actually measuring individual repair capacity was hovering there since the late 1970s, when it was noticed that there was significant inter-individual variation in the responses of cultured human lymphocytes to DNA damage induced by chemical agents (N-acetoxy-2-acetylaminofluorene and 7,12-dimethylbenz(a)

anthracene, adduct-forming agents) (64). The time was still not ripe, however, for making the connection between the subtle variance in capacity for repair of DNA damage, the risk for multifactorial disease and the possible outcomes of treatment, mainly because anticancer therapy itself was still very young, relying primarily on empirical knowledge, and the concept of multifactorial pathogenesis of late-onset diseases was not fully developed yet. Occasional reports about unusual genotype–phenotype correlations such as the paper of Fujiwara et al. (30) about generally healthy individuals carrying mutant alleles of crucially important genes that would have normally caused severe genetic disease were regarded as pure artifacts or, at best, as scientific curiosities. It was not until 1990, when Ara et al. (5) reported two polymorphic forms of the master regulator gene *TP53*, none of which could be associated with any distinct disease phenotype. The polymorphism (Pro72Arg, rs1042522) affected the coding sequence of the gene, substituting a proline residue for an arginine residue. As the protein encoded by the *TP53* gene, the transcription factor p53, plays so important a role in the life of the cell, it could be expected that virtually any mutation in the coding portions of the gene would be associated with genetic disease or, at least, distinct phenotype. Almost 10 years after the initial report of Ara et al. (5), in 1999, Thomas et al. (80) stated that both alternative forms of p53 ought to be considered wildtype, as they were conformationally indistinguishable and exhibited comparable DNA-binding affinities. The p53 (Pro) variant, however, was found to be a stronger transcription activator than p53 (Arg), whereas p53 (Arg) induced apoptosis more effectively than p53 (Pro). This finding was followed by the results of Khan et al. (44), published in 2000, about a benign polymorphism (insertion/deletion type) in a gene coding for a key protein of nucleotide excision repair, defects in which were previously found to be implicated in the most common form of xeroderma pigmentosum, XP-C. One year later, a common polymorphism in the *XPG* gene (His1104Asp, rs17655) was identified (27). It took, however, a couple more years to unravel the relationships between the carriership of polymorphisms in DNA repair genes and the risk for development of various cancers (35, 47, 57, 65); response to treatment and patient survival (45, 74, 92) and the risk for therapy-related complications (acute and delayed toxicity and risk for development of resistance to anticancer drugs) (4, 18, 68).

Effects of individual repair capacity status in health and disease

A 'polymorhic allele' is, by definition, an allele distinguishable from the allele most often seen in the particular locus, observed with a frequency of >5 % in the general population. ‚True' DNA polymorphisms have no direct effect on the phenotype of the carrier individual, that is, their presence is not readily detectable neither at the time of birth or in later life (e.g. by producing signs and symptoms associated with genetic disease or other readily discernible phenotype). Carriership of genetic polymorphisms is often completely asymptomatic, especially when the polymorphic alteration has occurred in non-coding parts of the gene and, sometimes, even in the coding sequence. Polymorphisms in genes coding for products acting in DNA repair and/or maintenance of genome integrity may constitute, at least partly, an exception to both rules. Some polymorphic alleles of genes of DNA repair are rare, with a frequency lower than 5 %, although many are quite common. Also, a decreased capacity for DNA damage repair in an individual is often associated with increased risk for development of various diseases and conditions, related to the accumulation of unrepaired DNA damage, e.g. insulin resistance, atherosclerosis, cardiovascular disease, and, of course, cancer. As in all multifactorial conditions, the sole presence of the factor does not translate immediately to imminent disease, but the carriers of the polymorphic allele/s are at greater risk for development of the associated conditions than non-carriers. This risk may be modifiable by environmental factors (e.g. smoking, UV exposure, or exposure to other environmental carcinogens).

Regardless of the strong genetic component in the capacity for DNA repair, it is not a fixed trait and its efficiency may significantly vary. For example, DNA repair capacity may be different in different phases of the life cycle (for instance, it is well known that the capacity for DNA repair generally declines with aging). Repair capacity has also been shown to vary between different types of cells. For example, it has been reported that cells of rapidly proliferating rodent tissues (e.g. liver, kidney and testis) show higher repair capacity by nucleotide excision than cells from slowly proliferating tissues (heart, skeletal muscle and lung) (36). Rapidly cycling cells rely heavily on global genome repair as they need to keep the integrity of their their DNA in check so as to proceed unevenfully with cell division. Rodent cells, however, possess unique traits that are not usually found in other mammalian cells. For example, rodent cells are usually deficient in the p53-dependent pathway of initiation of global genome repair (GGR) and focus on trancription-coupled repair (TCR), with the possible exception of tissues with rapid natural turnover. In human cells, it has been found that different types of DNA lesions may be repaired at differential rates by the different sub-mechanisms of nucleotide excision repair, with lesions in transcribed regions being repaired at similar rates, whereas lesions in untranscribed regions are repaired by GGR with varying efficiency, depending on the type of lesion (8). Specific subtypes of repair or general DNA repair may be physiologically suppressed in different types of non-transformed human cells (e.g. monocytes specifically inhibit DNA repair by base excision and repair of double-strand breaks in DNA) (9). Finally, transformed cells may express proteins that suppress DNA repair and induce chromatin hyperplasticity (e.g. wild-type or cancer-specific isoforms of HMGA1-group proteins) (2, 3).There is also the question of limitations of the methodology used to assess DNA repair capacity, as not all currently available methods are applicable to all types of damage. Usually, the capacity for repair of DNA damage is measured by indirect methods, evaluating

the degree of restoration of damaged DNA to its initial state (as it was before the damage occurred), which is reflected by the recovery of its sequence or structure and/or functionality. Some methodologies for assessment of DNA repair are virtually universally applicable for all types of DNA lesions (e.g. the hydroxyurea replication blockade and measurement of non-replicative DNA synthesis (19), while others may be better suited for detection of structural lesions in DNA, for example, the qPCR method (17), or double-strand breaks, e.g. the transformation efficiency assay described by Gospodinov et al. (37).

At present, the best studied associations between variant alleles of genes of DNA repair and risk of conditions with multifactorial pathogenesis are those for cancer. The individual repair capacity plays a role not only in the risk for development of cancer, but also in virtually every aspect of the course of the disease: the eligibility for certain types of treatment; the patient's survival (uneventful survival as well as overall survival) after therapy with specific agent/s, the possible adverse effects related to the treatment (acute and late toxicity) and the risk for development of resistance to therapeutic agent/s. The basic considerations associated with each of these aspects of individualised therapy of cancer are discussed in Part II.

Eligibility for specific treatment/s

The eligibility for treatment with specific agents is dependent on the balance between the expected survival (relapse-free as well as overall survival) of the patient, on the one hand, and the expected toxicity profile, on the other hand. When assessing the adequacy of treatment, even in modern medicine, one could only be truly objective in retrospective; that is, the 'right' therapeutic regimen had been selected of all possible treatments if the tumour responded to therapy *and* the adverse effects of the therapy were tolerable. Only the one without the other is unacceptable, since it causes needless suffering to human beings that are already ill, as more often than not the cancer is discovered when it is already locally or systemically advanced. With the intensive development of pharmaceutical science of today and the accumulation of knowledge about the mechanisms of development of different types of cancer, the task of choosing between different drugs is becoming more and more difficult. Some treatments that work well in many patients may only have limited effects in some patients, whereas others may have none at all. It is important to know whether a patient ought to be started on a certain drug at all, as a failed course may waste valuable time (an important factor in all diseases and specifically in advanced cancer) and may worsen the patient's condition.

It is important to note that the assessment of eligibility for treatment with some of the modern anticancer drugs is still sometimes based on purely empirical grounds. If the drug in question is known to have a potential for causing severe adverse effects, then patients that are elderly, already frail, or have a pre-existing condition that may be severely exacerbated by treatment with the prospective agent/s, may be evaluated as ineligible for the particular treatment because of their physical condition only. Other empirical factors, including lifestyle and habits are also sometimes taken into account. For example, the chemotherapeutic erlotinib, a tyrosine kinase inhibitor which suppresses the EGFR-related signaling in tumours of epithelial origin (non-small-cell lung cancer and, sometimes, pancreatic and colorectal cancer), was found to be less effective in smokers, because of accelerated clearance of the drug, resulting in less exposure of the tumour cells to the active substance (39, 72). The biochemical properties of the tumour may also give an indication about whether the patient is eligible for a certain type of treatment. For example, a triple-negative breast tumour (expressing neither the estrogen receptor alpha, nor the progesterone receptor or the HER2 receptor) is not likely to respond to estrogen receptor-targeted or HER2-targeted therapies). Therefore, patients with triple-negative tumours (e.g. invasive breast cancer) are usually started on genotoxic therapy without trials of antiestrogens (e.g. tamoxifen) and herceptin, which would very likely be ineffective (20).

Post-therapeutic survival

The survival in patients with cancer (measured in months and years of life after diagnosis) is dependent on many parameters. Some of these are related to characteristics of the tumour, e.g. its histological type, its localisation; the degree of vascularisation of the tumour tissue; the differentiation grade (poorly, moderately or well differentiated); its immunological and biochemical properties, etc. Others pertain specifically to the patient: their age; sometimes, their sex; their general condition; the hormonal status (e.g. in estrogen-dependent tumours in women, before or after menopause); the body mass index, the lifestyle and habits (e.g. smoking) and others. Since most cancer cells ultimately develop resistance to anticancer agents, the risk of induction of drug resistance during treatment is also a factor in a patient's survival, as the second-line anticancer agent may not be that effective as the first, to which, however, the tumour has become resistant. Eventually, the patient may die because of treatment-related adverse effects and not because of progression of the cancer.

Individual repair capacity affects virtually all factors that may play a role in the constitution of survival rates: sensitivity of the tumour to the chosen treatment, therapy-associated toxicity (tissue- and organ-specific or overall) and the risk of developing resistance mechanisms that allow the cancer cell to avoid, overcome or compensate the damaging potential of genotoxic therapies. Usually, the more damage a genotoxic compound causes to the tumour cells, the better the response to the treatment is in terms of tumour shrinkage and obstruction of the blood vessels of the tumour. This may be expected to be associated with superior survival rates, except in cases when the toxic effects on the healthy cells are so severe that they outweigh the beneficial effects of the drug (see Part II for details). If the tumour cells are well equipped to remove the damage from their DNA, or are capable of triggering specific mechanisms that would enable them to

remove this damage efficiently, they would be resistant to treatment or would be initially sensitive, then would acquire resistance. Usually, polymorphisms in DNA repair genes and genes coding for products responsible for maintenance of the genome integrity that **decrease** the capacity for repair of DNA damage are associated with better response to treatment (and, respectively, longer survival), as the cells would more rapidly accumulate suprathreshold levels of damage associated with induction of cell cycle arrest or programmed cell death. Sometimes, however, paradoxical responses may occur, with the polymorphic variant conferring higher capacity for DNA repair associated with superior survival (see Part II). This, however, is dependent on the type of tumour and the type of the genotoxic agent used.

Resistance to antitumour drugs

Some drugs used in anticancer therapy are metabolised by a designated enzymatic system and resistance to the drug may occur simply by up-regulation of the intratumoral expression of the respective enzyme. For example, steroid hormones as well as many antitumour drugs are substrates for the CYP1B1 enzyme of the cytochrome P450 family. Among the latter are genotoxic drugs, such as mitoxanthrone; mitotic inhibitors (docetaxel); antiestrogens (tamoxifen, flutamide), and others. In normal tissues, the CYP1B1 protein is expressed at low levels, but many primary and metastatic tumours over-express CYP1B1, which is associated with resistance to anticancer agents (33, 58). Cancer cells may also physically multiply the active gene copies coding for the enzyme or a key subunit of an enzyme, the expression of which is normally strictly controlled. Therefore, the synthesis of the enzyme used to degrade or inactivate the active compound is maintained at high levels in the tumour tissue. Such is the case, for example, with the dihydrofolate reductase (*DHFR*) gene, which encodes an enzyme that inactivates methotrexate and other cytostatic drugs (10). The effective intracellular concentration of some anticancer compounds may be drastically lowered by interaction of the compound with drug transporter proteins, e.g. multidrug resistance proteins (MDP) – transmembrane proteins capable of binding and transporting a variety of compounds outside the cell (reviewed in [63]). Various intracellular compounds (e.g. glutathione) may bind and inactivate anticancer drugs (81). Out of the wide variety of anticancer compounds, only platinum derivatives, due to their unique structure, are not 'metabolised' or 'biotransformed' (88). The genotoxic action of platinum derivatives is based on formation of adducts in DNA (dG–dG and dG–dA), mainly, between nucleotides in the same DNA strand, but also between different strands. Less commonly, platinum derivatives may cause DNA–protein crosslinks (96). The mechanisms of resistance to platinum compounds are not always related to simple modulation of enzyme activities, as the pathways of their biodegradation are different from these of most anticancer drugs. Unlike many drugs administered intravenously, platinum compounds are not rapidly bound to plasma proteins, but, rather, the platinum ions produced by spontaneous hydrolysis become bound to plasma proteins such as albumin, transferrin, and gamma globulin within 2–3 hours after intravenous administration, then the platinum-protein complexes are slowly cleared, predominantly by renal excretion, over the next several days. Resistance to platinum-based regimens, as that to all anticancer drugs, is dependent on mechanisms such as sequestering the active substance, routing it out of the cell or making it inactive or unavailable before it has found its target and implemented its functions. It is, however, strongly dependent on the capacity for excision of the drug-induced DNA adducts as well. It is not surprising, then, that most of the studies of the impact of individual repair capacity on drug resistance of tumour cells are carried out in patients treated with platinum derivatives. Indeed, platinum-based regimens (where the platinum derivative is used as a single agent or combined with other drugs) are used very often in treatment of solid tumours (because of the high response rates, comparable only to anthracycline-based regimens). Therefore, patients treated with platinum derivatives would be enrolled in clinical studies more often than patients treated with other types of genotoxic drugs. The ability of tumour cells to repair the damage inflicted upon their DNA by platinum compounds is dependent on their genetic background, constituted at least partly by the individual differences in the DNA repair capacity. Subtle as these differences may be, they may become significant under conditions of severe genotoxic attack produced by a therapeutic course with genotoxic agent/s. Cells with near-normal capacity for repair of DNA damage may be at lower risk for cancerous transformation, but once they have become transformed, they may repair therapy-induced genotoxic DNA damage effectively and may be, therefore, less sensitive to genotoxic treatments. Currently, strategies for sensitisation of cancer cells by decreasing their capacity to repair the damage inflicted by anticancer treatment are being intensively developed. Usually, these strategies are based on inactivation of a key protein acting in induction of cell cycle arrest and repair of damage in actively dividing cells. Among the common target proteins are, for example, ATM (inactivated by compounds such as KU-55933 and KU59403) (42, 43) and cyclin-dependent kinases (targeted by R-roscovitine [seliciclib], flavopiridol, difluoromethylornitine, and others) (7, 29).

Risk of toxicity in anticancer therapy

The individual tolerance to anticancer therapy is a rather complex issue, as the more aggressive a therapy, the better the chances for eradication of the tumour cells. Treatment with genotoxic agents, however, is always associated with risk of toxicity. Genotoxic treatments are targeted at rapidly cycling cells, as most of the cancer cells are, but affect, albeit to a lesser degree, all cells capable of division. Cells with a naturally rapid turnover (skin, hair, blood-forming tissue) are especially vulnerable. Preserved capacity for repair of DNA damage resulting from genotoxic treatments may be associated with poorer response to therapy, as tumour cells with repaired DNA are likely to restore rapidly their capacity for division. Near-normal DNA repair capacity in cancer

cells may, however, be associated with lower incidence or decreased severity of adverse reactions. The adverse effects from genotoxic therapy may sometimes actually outweigh the benefits of the therapy, as the quality of life of patients may be severely compromised. During or after treatments with genotoxic agents, some patients may become so ill that they may drop out of treatment, or, rarely, they may die because of severe inhibition of the growth and the functions of normal cells (e.g. agranulocytopenia, severe skin inflammation, or severe gastrointestinal disturbances). Toxicity is an anticipated effect in anticancer therapy and the goal is not to prevent it altogether (which is virtually impossible at present), but to decrease its severity and/or make its effects more tolerable, whenever possible. The prevalence of toxic effects related to anticancer therapy and their severity may greatly vary, depending on the type of the therapeutic agent/s used; but also on the individual characteristics of the patient. The latter includes general characteristics (age, general condition, body mass index); lifestyle traits (e.g. smoking, best studied in lung cancer patients that continue smoking after diagnosis) as well as purely individual characteristics, including the genetic background of the patient with regard to individual capacity for repair of therapy-inflicted damage, as well as other inherited polymorphisms, for example, in genes coding for factors acting in pro- and anti-inflammatory signaling and tissue renewal.

Toxicity effects may sometimes even be used as phenotypic markers for the response to genotoxic treatment. For example, the already mentioned EGFR inhibitor erlotinib causes rash in some patients. In about 10 % of all patients, the rash is severe (grade >2). Studies show that the appearance and the grade of the rash may correlate with the degree of response to therapy and patient survival in advanced cancer, with one-year survival being between 2 and 3 times higher in patients that had high-grade rash during treatment with erlotinib than in patients with low-grade rash or no rash at all (72).

Major players in the constitution of individual repair capacity

The assessment of individual capacity for repair of damage is often implemented by sets of markers, but it is much more than a simple sum of disparate markers. Nowadays, the number of markers for assessment of eligibility to various therapies grows by the week. Not all of these markers, however, offer reproducible results *in vitro*, and even less seem to be reliable enough *in vivo*. Several panels of markers have been tested so far, in patients with cancer as well as immunological diseases, with variable success (25, 41, 46, 55). At present, polymorphisms in about a dozen genes coding for major proteins of DNA repair and maintenance of genome integrity are considered reliably associated with significant effects on the phenotype in health and disease. The genes may be grouped by function as follows:

- Maintenance of genome integrity/induction of damage-related cell cycle arrest and/or apoptosis: *TP53* and *ATM*;
- Nucleotide excision repair: *XPA, XPC* (only in global genome repair), *XPD*, *XPG* (*ERCC5*) and *ERCC1*;
- Base excision repair: *XRCC1*;
- Mismatch repair: *MLH1* and *MSH6*;
- Repair of double-strand breaks: *XRCC2* and *XRCC3* (functioning in repair by homologous recombination) and *LIG4* (repair by non-homologous end joining).

Polymorphisms in most of these genes affect the susceptibility for development of different cancers or other multifactorial conditions, but their impact on the outcomes of genotoxic therapies and the risk for development of drug resistance has been studied thoroughly only for some of them. A summary of the currently available experimental information is presented below.

TP53

The *TP53* gene (17p13.1) codes for the master regulator protein p53, a transcription factor functioning in the regulation of the cell cycle, induction of aging and programmed cell death, regulation of cellular metabolism, etc. Inherited defects in the *TP53* gene are associated with Li-Fraumeni syndrome, a very rare genetic disease characterised by greatly increased risk for development of various cancers, of the common types (e.g. epithelial malignancies) as well as tumours rarely seen in the general population (usually, soft tissue sarcoma) (50). Over 200 naturally occurring variants of the *TP53* gene have been reported so far, germline and somatic, characteristic of cancer cells or occasionally seen as normal variants (89). Over-expression of cancer-specific p53 isoforms is usually associated with poorer prognosis for the patient (52, 53). Presence of alterations in the *TP53* gene in human tumours is associated with loss of capacity for induction of cell cycle arrest and apoptosis at the G1/S checkpoint (84). The *TP53* gene locus or the chromosome arm containing it may be selectively lost in tumours.

Two polymorphisms in the *TP53* gene specifically attracted the attention of researchers and clinical specialists, as they are not associated with a distinct disease phenotype, but may modify the risk for developing different conditions (including cancer) and the outcomes of various therapies. These are the already mentioned Pro72Arg polymorphism in exon 4 (5) and a 16 bp duplication in intron 3 (rs17878362) (93). The duplication polymorphism is presently much less well studied than the Pro72Arg polymorphism, but that is mainly because the Pro72Arg was identified 12 years earlier than the identification of the duplication in intron 3.

It was initially believed that carriership of the Arg allele of the Pro72Arg polymorphism could only be beneficial with regards to the risk of development of cancer and response to anticancer therapies. Indeed, it makes perfect sense that cells that exhibit natural propensity for induction of apoptosis would be more effective at triggering programmed cell death at early phases of neoplastic transformation, preventing development of overt cancer. Also, apoptosis-prone cells

treated with genotoxic agents were believed more likely to die when overloaded with DNA damage, instead of inducing cell cycle arrest, repairing the damage, then continuing with division. As it turned out, however, not only carriership of the Arg allele was not always associated with eradication of the tumour after genotoxic treatments, but the Arg allele might be preferentially retained and mutated in cancer cells (see Part II for more details).

The duplication allele is associated with decreased levels of p53 mRNA, resulting in less efficient activation of the p53-associated pathways for induction of cell cycle arrest, DNA repair and apoptosis (32). Carriership of the intron 3 duplication polymorphism in the *TP53* gene may increase the risk for breast cancer (91).

ATM

The *ATM* (ataxia-telangiectasia mutated) gene (11q22.3) codes for the ATM protein, responsible for induction of cell cycle arrest in response to DNA damage and DNA repair-associated signalling. ATM may reroute the cell's programme to apoptosis even if the p53-dependent apoptosis pathway is non-functional for some reason. Inherited defects in the *ATM* gene in a homozygous state are associated with ataxia-telangiectasia, a genetic disease characterised by progressive cerebellar ataxia, conjunctival telangiectasias and immune deficiency. Carriers of one defective *ATM* copy, albeit generally asymptomatic, are at increased risk for development of various cancers, specifically of the breast, the lung, the colon and the pancreas (31, 66, 71, 78). Polymorphisms in the *ATM* gene may be associated with increased risk for development of myelodysplastic syndrome (70). The rs189037 (G-to-A) substitution in the promoter of the *ATM* gene does not disrupt the function of the gene product but may be associated with male infertility due to idiopathic nonobstructive azoospermia (51). Homozygocity by any of the two intronic polymorphisms IVS22-77 T-to-C (rs664677) and IVS48+238 C-to-G (rs6094) and by one polymorphism in the coding sequence (G5557A, rs1801516) are associated with increased risk for breast cancer (6).

XPA

The *XPA* gene (9q22.33) encodes the XPA protein, which is part of the XPA-RPA complex that binds to damaged DNA in the early stages of repair by nucleotide excision (82). The XPA-RPA complex binds to the XPF-ERCC1 heterodimer and stimulates the endonuclease activity of the XPF and XPG proteins (69, 85). Several relatively common single-nucleotide polymorphisms were described in the *XPA* gene, for two of which, (Arg228Gln [rs1805160] and Val234Leu [rs3176749]) no significant differences in the DNA repair capacity or survival of cells carrying the one or the other allele were found (59). One polymorphism (rs1800975; an A-to-G substitution) in the 5′-untranslated region of the *XPA* gene (13) was found to be associated with **decreased** risk for lung cancers, even in individuals with history of exposure to tobacco smoke (94).

XPC

XPC is a protein coded by the *XPC* gene (3p25.1). It is a component of the XPC-hHR23 complex, which is the first factor binding to the damage site in global genomic repair (GGR), recruiting the other NER proteins. XPC is not involved in transcription-coupled repair, where the presence of stalled RNA polymerase II at the damage site is a sufficient signal for initiation of repair. The XPCins83 polymorphism is a composite alteration in intron 9 of the *XPC* gene, consisting of insertion/deletion of 83 bp poly-AT region, in linkage disequilibrium with a 5 bp deletion (44). The insertion allele is associated with decreased capacity for detection of DNA lesions in untranscribed genomic regions and increased risk for some tumours, such as lung cancer, esophageal cancer, melanoma and others (11, 16, 48, 57). The XPCins83-associated cancer risk may be modified by other factors, genetic as well as environmental (e.g. smoking) (67). Two more polymorphisms in the coding sequence of the *XPC* gene (Lys939Gln [rs2228001] and Ala499Val [rs2228000]) were described (45), but their involvement in susceptibility to cancer has not yet been definitely proven.

XPD

The *XPD* gene (19q13.32) codes for one of the two DNA helicases (XPB and XPD) unwinding DNA at damage sites in order to permit the access of the NER machinery. Several polymorphisms in the *XPD* gene were described, Arg156Arg (rs238406, a synonymous substitution of C with A), Asp312Asn (rsl799793) and Lys751Gln (rs1052559), associated with increased risk for prostate carcinoma, breast carcinoma, lung and head and neck cancers (56, 77). The risk for lung cancer conferred by polymorphic variants in the *XPD* gene is modifiable by environmental factors, specifically, smoking (55). Carriers of the 751Gln variant may be susceptible to development of low-risk colorectal adenomas (76). The carriership of polymorphisms in the *XPD* gene is also associated with increased risk for a non-cancerous late-onset disease, namely, senile cataract (60).

XPG (ERCC5)

XPG (ERCC5) is a protein with endonuclease activity acting at the late stages of NER, cleaving the repaired strand in the 3'-direction from the damage site (38). Several polymorphisms have been described in the *ERCC5* gene (13q33.1), but only the synonymous His46His (rs1047768) and His1104Asp substitutions (rs17655) were found to be associated with significant effects on the phenotype. Carriership of the Asp variant of the His1104Asp polymorphism may be associated with **decreased** risk for cancer of the pharynx, the oesophagus, and the lung (23).

ERCC1

The *ERCC1* gene (19q13.32) codes for one of the subunits of the ERCC1-XPF nuclease complex, playing a role in the late stages of NER (regardless of the type of induction of

repair), introducing the 5'-strand break in DNA. Several polymorphisms have been described in the *ERCC1* gene, but the C8092A (rs3212986) polymorphism in the 3'-untranslated region of the gene is the best studied one at present. The C allele of the C8092A polymorphism is associated with lower transcript stability (90). Carriership of the *C8092A* polymorphism of *ERCC1* may be associated with increased risk for adult glioma (21) and tumours of the head and neck (77, 95). The CT heterozygotes by the T19007C polymorphism (rs11615) in *ERCC1* were reported to be at increased risk of development of skin cancer (97). In the same study the *ERCC1* 17677A (rs3212961) polymorphism was found to be associated with increased overall risk of cancer, without specification of the type of tumour.

XRCC1

The *XRCC1* gene (19q31.1) codes for a stabilising factor of ligase III, which is the primary ligase of base excision repair (14). Over 20 non-synonymous polymorphisms have been described in the *XRCC1* gene, of which clinical significance has been found for Arg194Trp (rs1799782), Arg399Gln (rs25487), Arg280His (rs25489) and His107Arg (rs2228487) (1, 75, 79). The impact of the *XRCC1* polymorphisms may be different in different populations. For example, the His allele of the *XRCC1* Arg280His polymorphism was reported to be associated with increased risk for development of colorectal adenoma in the Norwegian population, while the 399Gln variant was associated with **decreased** risk for development of high-risk adenomas (76). However, carriership of the 399Gln and 194Trp variants was found to be associated with early-onset colorectal carcinoma in the Egyptian population (1). Carriership of polymorphisms in the *XRCC1* gene is another genetic factor associated with increased risk for senile cataract (60). The Arg399Gln polymorphism is associated with susceptibility to endometriosis (40).

XRCC2 and *XRCC3*

XRCC2 (7q36.1) and *XRCC3* (14q32.33) are members of the *RAD51* gene family, coding for proteins involved in repair by recombination. Specifically, the XRCC2-XRCC3-RAD51 complex ensures the uneventful migration of the cruciform structure in homologous recombination and the subsequent resolution of the recombinant molecules (54, 61). Homozygous carriers of the variant allele of three polymorphisms in the 5'-noncoding UTR of the *XRCC2* gene (rs10234749 C-to-A, rs6464268 T-to-C and rs3218373 G-to-T) and one in the coding sequence (Arg188His, rs3218536) are at **reduced** risk for bladder cancer compared to carriers of the more common allele (28). The Arg188His polymorphism in the *XRCC2* gene and the XRCC3 Thr241Met (rs861539) polymorphism have been recently identified to be associated with increased risk for head and neck cancer (86).

In vitro assays in peripheral lymphocytes from clinically healthy human volunteers show that the polymorphisms Lys751Gln in the *XPD* gene, Asp1104His in the *XPG* gene, Lys939Gln in the *XPC* gene, Arg399Gln in the *XRCC1* gene and Thr241 Met in the *XRCC3* gene cause increased levels of strand breaks and chromosomal aberrations (83).

MLH1

MLH1 is a human MutL homologue, functioning in the early phases of mismatch repair. Inherited mutations in the *MLH1* gene are associated with greatly increased susceptibility to hereditary non-polyposis colorectal carcinoma (HNPCC) (12). The *MLH1* -93 G-to-A polymorphism was found to be associated with increased risk for colon cancer, but the risk was not as high as in HNPPC (15). Therefore, the -93 G-to-A substitution was classed as a polymorphism rather than a deleterious mutation. Several substitutions of non-conserved amino acid residues were described in the *MLH1* gene – Val219Ile, Ser406Asn and Lys618Ala. These polymorphisms were initially believed to be deleterious mutations, associated with HNPCC (62), but were later classed as neutral substitutions. Some of these polymorphisms may be associated with toxicity effects in patients treated with ionising radiation (24) (for more information, see Part II).

MSH6

The *MSH6* gene (2p16.3) codes for one of the six homologues of the prokaryotic MutS protein (MSH). Human MSH6 protein usually functions as part of a heterodimer with the protein MSH2 (hMutSα), recognising single-base mismatches in an ATP-dependent process (reviewed in [49]). MSH6 is part of the BRCA1-associated genome surveillance complex (BASC), together with other DNA repair proteins, among which are BLM (Bloom helicase), ATM, MSH2, MLH1, replication factor C (RFC) -1, 2 and 4, and the MRN (MRE11- RAD50-NBS1) complex that is involved in recognition and repair of structural damage in DNA (87). Mutations in the *MSH6* gene are identified in a proportion of cases of familial non-polypous colorectal cancer (26). The *MSH6* polymorphism Gly39Glu (rs1042821) is associated with risk of colon cancer (15). The risk is modifiable by dietary habits (e.g. Western pattern diet) and smoking.

LIG4

The *LIG4* gene (13q33.3) codes for ligase IV, a protein responsible for ligation of free ends in repair of double-strand breaks by non-homologous end joining (NHEJ) as well as in V(D)J recombination. Inherited defects in *LIG4* are associated with several inborn disorders characterised by immune deficiency and hypersensitivity to ionising radiation. Carriership of some of the allelic variants of the *LIG4* gene may be associated with **decreased** risk for multiple myeloma (73). Three polymorphisms in the *LIG4* gene – Thr9Ile (rs1805388), Ile658Val (rs2232641) and a synonymous Asp568Asp polymorphism (rs1805386), were recently found to be implicated in the susceptibility to head and neck cancer (86).

Conclusions

Individual capacity for repair of damage in DNA may vary even between healthy individuals. Carriership of polymorphisms in genes coding for products functioning in recognition of DNA damage, damage-associated signaling and DNA repair may play a role in the constitution of the risk for development of many common late-onset diseases, and, specifically, cancer. As modern anticancer therapy is based mainly on infliction of DNA damage in rapidly proliferating cancer cells, decreased capacity for recognition and repair of DNA damage may modify the outcomes in cancer patients with regard to therapy response, post-therapeutic survival, acute and late toxicity, and risk for development of resistance to anticancer drugs. This two-part paper reviews the currently available data about the impact of carriership of polymorphisms in genes of DNA damage recognition and repair on the constitution of risk for common cancers and the outcomes of genotoxic therapies.

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