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DNA Repair Capacity and the Risk of Head and Neck Cancer

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1. Introduction

According to the chemical carcinogenesis hypothesis (Husgafvel-Pursiainen, 2004) (Loeb & Harris, 2008) chemical carcinogens exert their activity by penetrating cells, cell nuclei and interact with DNA generating DNA lesions such as single- or double-strand DNA breaks and DNA base modifications (adduction, substitution, base oxidation). DNA lesions when not removed are fixed as DNA mutations. Mutations as alterations of DNA structure are responsible for a change of the genetic information and the same remain the key step in carcinogenic transformation of cells. The whole process of carcinogens metabolism in a cell proceeds under enzymatic control (Fig.1). The first step known as metabolic activation provides changes in carcinogen molecule into better solubility and higher reactivity with detoxifying enzymes. Only few chemical carcinogens can omit this step and react directly with the enzymes. The second step being a detoxification proper consists of enzymatic conjugation of activated carcinogens and removal carcinogen metabolites out of a cell. This step is highly efficient and usually over 90% of exogenous compounds are being removed. At this stage the activated carcinogens are capable to react with DNA but still detoxification remains the main pathway when a reactivity towards DNA is less likely. Generation of DNA lesions is automatically followed by DNA repair.

The process of DNA repair is recognized as the last line of a cell defense against mutagens/carcinogens. DNA repair is carried into effect on separate pathways adjusted to a type and extent of DNA damage, and the cell cycle (Jenkins et al., 2010). A most commonly accepted categorization of DNA repair mechanisms is shown in Table 1. To comment a multitude of DNA repair mechanisms, a direct repair, DNA excision repair and recombination repair are specialized in dealing with various types of DNA lesions at first instance. Mismatch DNA repair pathway works later to remove DNA lesions remaining after processing by the above mentioned mechanisms or emerge late in the cell cycle. Recombination repair is involved both in DNA and chromosome repair. SOS-repair acts only when DNA lesions occur abundantly.

There are roughly 4000 chemical compounds occurring in tobacco smoke. Carcinogenic properties were attributed up to 60 constituents carcinogenic properties. The main
carcinogens of tobacco smoke are: polycyclic aromatic compounds (with benzo/a/pyrene as the best known example), aromatic amines, N-nitrosamines and reactive oxygen species (Talhout et al., 2011). The molecular aspects of carcinogenesis generated by tobacco smoke is relatively well recognized (Hecht, 2003; Husgafvel-Pursiainen, 2004). Lesions generated by tobacco smoke carcinogens include single-strand DNA breaks, adducts carcinogen:DNA and oxidative DNA damage. Any preference to a specific DNA sequence was not established. Anyway, guanosine residue was found to be the most sensitive DNA element for carcinogen attack. Regardless a lack of sequence specificity an attention was focused on lesions generated in cancer-related genes. TP53 lesions and mutation were studied most efficiently. A direct relation between benzo/a/pyrene:guanosine adducts formation generating later on G:C to T:A mutations was proven in the case of TP53 (Denissenko, 1996). Further, studying cancer-related genes a pattern of mutations derived from tobacco smoke exposure was identified in TP53 and other genes (Le Calvez et al., 2005).

Fig. 1. Carcinogen metabolic pathways and health effects.

An epidemiological evidence for a causative contribution of tobacco smoking to cancer of lung or larynx as well as an increased risk to develop many types of cancers among tobacco smokers has been provided long ago (Vineis et al., 2004). A strong association between tobacco smoking and cancer risk leads to an opinion on predominance of exogenous factors over genetic background (Le Marchand, 2005). On the other hand, only a minority of smokers develop any type of cancer. Hence, it was deduced that a combination of exogenous exposure and individual predisposition is responsible for carcinogenic transformation (Taioli, 2008). An extensive review of cancer genes divided into contributing
Table 1. Classification of DNA repair pathways.

<table>
<thead>
<tr>
<th>Type</th>
<th>Subtype</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct repair</td>
<td>Restricted to a few lesions</td>
<td></td>
</tr>
<tr>
<td>Excision repair</td>
<td>Base excision repair (BER)</td>
<td>Removes majority of lesions</td>
</tr>
<tr>
<td></td>
<td>Nucleotide excision repair (NER)</td>
<td>As above</td>
</tr>
<tr>
<td>Recombination repair</td>
<td>Homologous end joining</td>
<td>Repair of DNA and chromosomes</td>
</tr>
<tr>
<td></td>
<td>Non-homologous end joining</td>
<td>As above</td>
</tr>
<tr>
<td>Mismatch repair</td>
<td>Specialized in removal mispairing of DNA bases</td>
<td></td>
</tr>
<tr>
<td>Transcription-coupled repair</td>
<td>Preference for actively transcribed genes, fast</td>
<td></td>
</tr>
<tr>
<td>Global repair</td>
<td>Slow repair of overall genome</td>
<td></td>
</tr>
<tr>
<td>SOS repair</td>
<td>Mutation-prone system</td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>Homologous recombination</td>
<td>ICL removal</td>
</tr>
</tbody>
</table>

to cancer risk and connected with cancer progression was written by Vogelstein and Kinzler (2004). An impact of genetic factor on health effects of tobacco smoking was also discussed more specifically in relation to head and neck cancer (Badawy et al., 2008; Rossing, 1998). As such specific genes as BRCA1 or APC involved in breast and colon cancer, respectively, were not discovered in head and neck cancer, an attention was paid on so-called “low-penetration” genes, mainly those involved in carcinogens processing. Within the group of low-penetration genes a potential significance of DNA repair genes was admitted (Livneh et al., 2005). The latter author points at the key function of DNA repair that is removal or bypass the damaged sites to restore the original structure and function.

2. Genotype versus phenotype

A comparison of tumor cells with normal cells has shown that the level of damage is higher in neoplastic cells. An observation was applicable to various types of damage including single-strand DNA breaks, carcinogen:DNA adducts, DNA mutations, chromosome aberrations and others. Next, an observation was extended also on other cells including peripheral blood lymphocytes derived from cancer subjects compared to those from healthy individuals. The latter observation has got a substantial significance as blood lymphocytes are commonly used as a surrogate material for biological analysis obtainable on non-invasive way (Phillips, 2005). However, the early results concerning a high level of DNA lesions and chromosome aberrations in cancer subjects posed a fundamental question concerning a role of former exposure to carcinogens confronted with an impaired DNA repair. Although some papers ignored an impact of DNA repair, their authors brought into light a differentiation between active and passive smoking as a source of carcinogens (Phillips, 2002), location of an organ exposed (Hainaut, Olivier, & Pfeifer, 2001) or exposure threshold (Jenkins et al., 2010).
On the other hand an early paper from M.R. Spitz group (Wei, Cheng, Hong, & Spitz, 1996) dealt with DNA repair in lung cancer subjects. DNA repair capacity was estimated in peripheral blood lymphocytes using host-cell reactivation assay, which measures cellular reactivation of a reporter gene damage to exposure to activated benzo/a/pyrene. The mean level of DNA repair was significantly lower in subjects than in controls. The final conclusion was that individuals with reduced DNA repair level are at an increased risk to develop lung cancer. Applying the same method the study was repeated on head and neck subjects. A reduced DNA repair capacity was shown when 55 newly diagnosed, untreated head and neck subjects were compared to 61 healthy controls (Cheng et al., 1998).

Following years have provided several papers documenting impairment of DNA repair potential in association with cancer. Alternative methods were shown to be useful to study DNA repair impairment. Schmezer et al. (2001) applied comet assay to study bleomycin-induced damage in peripheral blood lymphocytes. Lung cancer blood donors (100 cases) were found more sensitive for such damage in comparison with healthy donors (110). High mutagen sensitivity in cancer subjects was explained as resulted from significantly reduced DNA repair capacity. The paper contains also interesting technical remarks. The authors demonstrated applicability of cryo-preserved lymphocytes to estimate DNA repair up to 12 months after blood collection. A similar experimental attempt was used in our own studies (Gajecka et al., 2005). A higher level of spontaneous and benzo/a/pyrene-induced was shown by comet assay in peripheral blood lymphocytes derived from laryngeal cancer subjects compared with healthy donors (52 cases v. 54 controls). A level of spontaneous DNA damage tended to increase with tumor grading. The latter result was not confirmed by the analogical study (L. E. Wang et al. 2010) done on much bigger group (744 head and neck subjects v. 753 matched cancer-free controls). The conclusion of Wang et al. was that the reduced removal of tobacco smoke-induced DNA adducts contributes to the risk of head and neck cancer but does not influence tumor characteristics. However, our results taken as "phenotype" were apparent. Then, we attempted to explain them by distribution of so-called “risk” and “protection” genotypes of XPD, XRCCI and XRCC3 genotypes of DNA repair genes. The distribution differences did not reach the level of statistical significance that means that phenotype-genotype correlation was poor. Still, phenotypic deficit of DNA repair was only partly confirmed by overrepresentation of “risk” genotypes of the studied DNA repair genes. Again, an impairment of DNA repair was shown in head and neck cancer cells taken from tissue biopsies compared to peripheral blood lymphocytes of healthy controls exposed to hydrogen peroxide treated taken as a model genotoxicant (47 cases v. 38 healthy controls). An advanced comet assay permitted for estimation of single-strand DNA breaks, oxidative lesions and efficiency of DNA repair. Besides, a general deficit of DNA repair, the authors established that H2O2-induced lesions were repaired less effectively in cancer cells from patients with metastasis than from those without metastasis (Rusin et al., 2009).

The most direct way to understand a link between DNA repair deficit and head and neck cancer are studies on processing of tobacco smoke lesions and their removal (Hecht, 2003; L. E. Wang et al., 2010; Wei et al., 1996). Polycyclic aromatic hydrocarbons are represented by several compounds present in tobacco smoke and generate a variety of carcinogen: DNA adducts. The structural features of the PHA-adducts play a role in differential repair of these adducts. Although PAH:DNA adducts are repaired dominantly by nucleotide excision
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repair (NER) pathway, differences in repair efficiency still occur for individual DNA adducts removal (Zhong et al., 2010). At this point it is necessary to remind that DNA lesions not removed fast enough are most harmful for a cell.

It was already mentioned that another type of DNA lesions generated by tobacco smoke is oxidative DNA damage. The major mechanism for repair of oxidized DNA bases represented by 8-oxoguanine is base excision repair (BER), but nucleotide excision repair seems to be involved too. It has been shown in lung cancer that the enzymes involved in recognition of oxidative DNA damage alter their activity and, in turn affect tumor growth because of increased formation of apurinic sites in DNA (Radak et al., 2005). The question of alterations of oxidative DNA damage repair which leads to accumulation of oxidative DNA lesions, mutagenesis and cancer development was reviewed recently (D’Errico, Parlanti, & Dogliotti, 2008).

In head and neck cancer one of the most disregulated genes is epidermal growth factor receptor overexpressed in ca. 90% cases. Reiter et al. (2010) undertook a study to explain a reason of EGFR overexpression in terms of sensitivity to mutagens and DNA repair potential. Oropharyngeal cancer cells were exposed to activated benzo/a/pyrene in vitro. Unexpectingly, an extent of damage was comparable in EGFR gene locus and in chromosome centromere, as well as in non-tumor tissue control. Also DNA repair capacity was comparable in all experiments. It looks, nor EGFR gene amplification, neither DNA repair might have a moderate effect on protein over-expression.

3. Evidence from genetic polymorphism

Variation in DNA repair as a factor in cancer susceptibility was associated early with single nucleotide polymorphism (SNP). Many gene variants are common in human population. Some structural variants decreasing a capacity to repair DNA were found more frequently in cancer cases and could be recognized as “risk” variants. The extreme case of DNA repair deficiency was established in a few rare diseases where DNA repair defect was followed by over-frequent tendency to develop cancer (Tab. 2). Gene variants working opposite way would be known as “protective” variants. Such attribution is not having a universal character because of ethnic differences in gene variants distribution (Becker, Nieters, & Rittgen, 2003; Mohrenweiser & Jones, 1998). Though, the mechanisms dominantly involved in their repair of tobacco smoke-induced lesions are: base-excision repair (BER), nucleotide excision repair (NER) followed by recombination repair and mismatch repair, it was assumed that impairment of DNA repair process, particularly in the key pathways would contribute to the increased risk of head and cancer. This assumption is workable within the hypothesis of chemical carcinogenesis but a connection with the hypothesis on cancer stem cells (Sales, Winslet, & Seifalian, 2007) is not excluded.

At this point one had to stress that the working material for studies on genetic polymorphisms are blood cells as all the cells of the organism have the same genetic information not changed throughout their ontology.

Establishing a link between single nucleotide polymorphism, distribution of gene variants and cancer risk was followed by publication of plethora of papers. Very early papers deal usually with an involvement of one repair gene/enzyme. The good example is a paper of
Table 2. Major diseases associated with DNA repair deficit.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Main genes identified</th>
<th>Cancer preference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xeroderma pigmentosum</td>
<td>XPA, XPC, XPD, XP-variant</td>
<td>skin</td>
</tr>
<tr>
<td>Ataxia telangiectasia</td>
<td>ATM</td>
<td>breast, other sites</td>
</tr>
<tr>
<td>Cockayne’s syndrome</td>
<td>ERCC6 (CSB), ERCC8</td>
<td></td>
</tr>
<tr>
<td>Bloom’s syndrome</td>
<td>BLM</td>
<td>Broad spectrum of cancer predisposition</td>
</tr>
<tr>
<td>Fanconi’s Anemia</td>
<td>14 FA genes (listed below)</td>
<td>AML, head and neck, breast, gynaecological cancers</td>
</tr>
<tr>
<td>Trichothiodystrophy</td>
<td>XPD</td>
<td>skin</td>
</tr>
</tbody>
</table>

Sturgis et al., (1999) describing an analysis of X-ray cross-complementing group one (XRCC1) gene polymorphism in relation to head and neck risk. The XRCC1 gene participates in base excision repair (BER) by encoding a protein acting in the repair of single-strand DNA breaks. Two different polymorphisms of XRCC1 were studied in the group of 203 cancer cases v. 425 controls. Distribution of gene variants did not vary considerably between the groups but XRCC1 26304T allele was found as a risk factor. The same group (Ho et al., 2007) studied also polymorphism of the same gene as a risk factor of salivary gland carcinomas. Three groups of samples were collected: 138 salivary gland carcinomas, 50 benign gland tumors and 503 cancer-free controls. It was established that XRCC1 1915C allele was associated with a lower risk and XRCC1 194Trp allele with a higher risk of salivary gland carcinoma. A much broader character had a study of Hao et al. (2004) on gene polymorphism connected with BER mechanism of DNA repair. There have studied 129 SNPs in the eight BER genes including ERCC1. An estimation of distribution of gene variants in Chinese cohort (419 esophageal cancer subjects v. 480 healthy controls) pointed at four gene variants including XRCC1-77C allele as predictors of an increased risk. Esophageal cancer does not belong formally to head and neck cancer group but anatomical vicinity and histological classification as squamous cell carcinoma allows for a close analogy. Interestingly, analogical on a large cohort of breast cancer (over 20 000 cases v. comparative number of controls) established only a moderate influence of one of three studied polymorphism on breast cancer in Asians and Africans but not in Caucasians (Y. Huang, Li, & Yu, 2009).

Investigation of gene polymorphism in nucleotide excision repair (NER) attracted many research groups. The studies were focused on XPD that is an evolutionary conserved ATP-dependent helicase, responsible for unwinding DNA molecule and removal of bulky carcinogen:DNA adducts. Structure-activity relationship of polymorphic gene variants of XPD was already established and described with considerations on its impact of cancer risk (Lunn et al., 2000). Previously shown reduced DNA repair capacity in head and neck cancer subjects together with removal of tobacco smoke-derived DNA adducts by XPD allowed to expect a key role of this gene in risk modulation. In line with this expectation Sturgis et al. (2000) studied a distribution of two polymorphisms of XPD in 189 head and neck subjects and 496 cancer-free controls. All subjects were non-Hispanic whites. A frequency of XPD 22541A variant did not differ enough to influence a relative risk. For XPD 39531C a
moderate increase of risk was established. The latter polymorphism was studied also on Swedish cohort consisting of 185 lung cancer cases v. 162 matched controls. The conclusion of the study was that the XPD variant alleles may by associated with a reduced repair of aromatic DNA adducts and increase lung cancer risk (Hou et al., 2002). The results of Hou et al. were fully confirmed in another study done on Chinese cohort (135 cancer cases v. 152 controls) targeting for esophageal squamous cell carcinoma (Yu et al., 2004). However, a meta-analysis of genetic polymorphism of three genes involved in NER (namely: ERCC1, XPD and XPG) provided some skepticism about their role. The authors concluded that fluctuation of variant frequency and their association with cancer risk will probably be minimal (Kiyohara & Yoshimasu, 2007). As the published results did not meet expectation it attracted to search for other polymorphism not known yet. An example is the paper by Kumar et al. (Kumar, Angelini, & Hemminki, 2003), who discovered a novel intronic mutation in the XPD gene.

Hence, further studies were aiming for a parallel coincidence of gene defect, gene-gene and gene-environment interaction. The genes most commonly studied for a joint effect were XRCC1, XRCC3 and XPD, representing different DNA repair pathways, namely: BER, NER and recombination repair, respectively. Such attempt was used by Matullo et al. (2003) to estimate effect of three polymorphisms in XRCC1 exon 10, XPD exon 23 and XRCC3 exon 7. Distribution of gene variants was assessed in 628 Italian healthy individuals and confronted with the level of aromatic DNA adducts in blood lymphocytes. A dose-response relationship between the number of risk variants and DNA adduct level. The interpretation was that the combined effect of multiple variant alleles modulates DNA repair capacity. The same trio of DNA repair genes supplanted by MGMT (enzyme coded by MGMT is repairing O\textsubscript{6}-alkylguanine adducts) was analyzed in 555 head and neck cancer cases v. 792 controls. Ethnic differences were found. XRCC1 Gln399Gln was associated with decreased risk in whites, but XPD 751 and XRCC3 241 did not change cancer risk. (W. Y. Huang et al., 2005).

Extension of a list of genes and their polymorphisms was done by De Ruyck et al. (2007) by an analysis (110 lung cancer v. 110 non-cancer controls) of 10 polymorphisms of genes involved in BER (XRCC1, APE1, OGG1, XPA, XPC and XPD). Primary significance was attributed to APE1 Asp148Glu, and the secondary one to XRCC1 ArgGln and XPD Lys751Gln concerning conversion of tobacco smoking effects into lung cancer. At this point it is necessary to admit the papers claiming no measurable effect of DNA repair genes polymorphism on cancer risk (London et al., 2001).

Because of a lack of convincing results concerning an impact of individual gene variants in cancer risk, recent years there is a tendency to study in parallel experiments a series of genes and multiple polymorphisms. Also a study groups tend to gain high numbers to get a reasonable statistic power. Within it a meta-analysis of previously published results plays an important role. An example of such situation is a paper Michiels et al. (Michiels et al., 2007) who studied polymorphism in 70 genes in lung cancer, head and neck cancer and healthy Caucasian individuals. None of the studied genes BER- or NER-associated genes was found to be associated individually with an influence of head and neck risk. Contrary, a few alleles of the genes attributed to DNA replication, translesion synthesis and transcription appeared to change a genetic risk. An international team anchored at International Agency for Research on Cancer, Lyon, France, presented an analysis of 28
SNPs in 18 DNA repair genes 9 SNPs in 7 cell cycle control genes on 811 cases with the upper aerodigestive tract v. 1083 individuals not related to tobacco and alcohol. In DNA repair genes only two variant alleles of MGMT and one allele of OGG1 were found moderately more frequently in cases, whilst only three alleles including XPA variant were associated with a protective effect. The authors discuss a false positive results present in papers based on small study groups an postulate further extension of a number of individual include into study (Hall et al., 2007). Under the same auspices another broad analysis was done in connection with the upper aerodigestive tract (Canova et al., 2009). Studying the material derived from 10 European countries including 1511 cancer cases and 1457 controls the authors analyzed 115 SNPs from 62 genes already known to be associated with cancer. In 22 SNPs of DNA repair genes only three were found statistically significantly reducing the risk. The results are meaningful as that time it was the largest genetic epidemiologic study on the upper aerodigestive tract in Europe. Almost the same time there was published a large analysis of genetic polymorphism in breast cancer (Smith et al., 2010). Distribution of gene variants derived from 18 SNPs was estimated in 336 cases v. 416 controls (all Caucasians). An impact of individual genes only three cases reached a level of significance. The authors suggest (i) to perform combine SNPs analysis (polygenic model) and (ii) to increase the sample size.

Altogether, the hypothesis of a significant impact of polymorphism of DNA repair genes besides many research efforts was not sufficiently proven but it does not eliminate DNA repair from cancer risk estimation. Its moderate effect was shown and a necessity of gene-gene interaction was suggested.

4. Specific types of cancer

To unravel a link between DNA repair and risk of cancer an alternative way of studies explores sub-classification of cancer to eliminate potential differences connected with etiology, histology, progression and other factors.

Rodriguez et al. (2007) restricted their interest to the early oral squamous cell carcinoma on one site, and on MGMT, that protects against alkylating mutagens repairing DNA in BER pathway. Working on protein level they observed a loss of MGMT protein expression from leukoplakia to early oral cancer. MGMT expression was declining also undifferentiation, tumor thickness and grading. Hence, MGMT expression relates rather to cancer progression than to risk. It also seems to indicate a poor prognosis. Unfortunately the study was done on a small sample. An involvement of MLH1 gene, being a part of mismatch repair pathway early stage of oral cancer, was studied by Gonzales-Ramirez et al. (2010). It was shown that gene expression is inhibited by promoter methylation that also located an involvement of this gene rather in tumor progression than in risk to develop cancer.

For such a late stage of oncogenesis as entering metastasis, Sarassin and Kauffmann (Sarasin & Kauffmann, 2008) put forward hypothesis on association of overexpression of DNA repair genes with metastasis. An assumption is that initial genetic instability need in time some genetic stabilization provided by over-expression specific DNA repair genes to invade and give rise to distant metastasis. To our knowledge the hypothesis has not been verified experimentally.
A risk of death for head and neck cancer is high for patients with second (multiple) primary tumors that encouraged clinical, molecular and genetic studies. Gal et al. (2005) were studying polymorphism of such DNA repair genes as XRCC1, XRCC3, XPD and MGMT in 279 subjects with already diagnosed and treated oral cancer who later on develop second primary tumors in upper aerodigestive tract. Polymorphism in XRCC3 241Met was found associated with an increased risk of second neoplasm. On the contrary XRCC1 399Gln gene was linked to a decreased risk of all-cause mortality in patients with oral cancer. In our studies we analyzed an impact of 11 polymorphisms of genes coding two activating enzymes, four detoxification enzymes and XPD, XRCC1 and XRCC3 DNA repair enzymes in 84 subjects with multiple primary tumors. The results were compared to that in 182 subjects with a single primary tumors and 143 cancer-free individuals. Looking at an impact of a single gene no correlation was found in case of DNA repair genes. The coexistence of some genotypes/alleles associated with higher cancer risk was established as an added factor for multiple primary tumor development. Our data indicate that the same group of low-penetration genes is involved in the development of single and multiple primary head and neck cancer, but their association with multiple primary tumor is significantly stronger (Rydzanicz, et al., 2005). Significance of NER core gene polymorphism was studied in the group of 1376 squamous cell carcinoma head and neck patients. Out of them 110 patients developed second primary tumors. None of seven studied genes had a pronounced effect for a risk to develop second primary tumors, that to some extent confirms our findings. However, the authors suggest that a profile of NER core gene polymorphisms might collectively contribute to the risk.

Nowadays the techniques exploring DNA microarrays technology became available and contribute well to the reviewed matter. An example of application of gene expression profiling of DNA repair genes is the paper of Rentoft et al. (2009) attempting to differentiate a pattern of DNA repair genes involved in development of head and neck cancer in young (<40 years) and elderly subjects. No genes were detected as significantly differentially expressed between young and elderly subjects.

5. Fanconi anemia pathway

Apart from previously described DNA repair systems present in human cells the Fanconi anemia pathway occupies equally important place. Fanconi anemia (FA) represents a specific and relatively rare syndrome, primarily described in 1927 by Guido Fanconi (Fanconi, 1927) (a Swiss pediatrician). He observed in 3 siblings hereditary form of aplastic anemia, panmyelopathy, short stature and hyper-pigmentation, nowadays syndrome known as Fanconi anemia. The FA is genetically and phenotypically highly heterogeneous condition, whereas patients display a wide variety of abnormalities. Some common features attributed to FA are multiple congenital malformations of skeleton and inner organs, radial ray defects, abnormal pigmentation and overall altered growth (Alter & Kupfer, 1993). However, the most important clinical feature are hematological abnormalities: bone marrow failure, aplastic anaemia, myelodysplastic syndrome (MDS), and high proneness to acute myeloid leukemia (AML). Besides AML FA patients also carry high risk of developing squamous cell carcinomas (SCC), especially of head and neck, gynecological and skin tumors (Alter, 1996; Lustig, et al., 1995, Rosenberg, et al., 2003). A considerable part of
cancer biology knowledge was described thanks to rare genetic syndromes related with cancer proneness such as Fanconi anemia.

Physiologically, patients with Fanconi anemia demonstrate high susceptibility to cross-linking agents, which result in significantly increased number of DNA damage events (DNA cross-links are most common). As the consequence repair capacity is insufficient to maintain DNA integrity. Consequently, the FA pathway is frequently deregulated or inactivated.

An increased sensitivity of FA patient-derived cells to the lethal effects of numerous cross-linking agents was described already over three decades ago in mid 70s (Ahmed & Setlow, 1978; Fujiwara & Tatsumi, 1975). Cloning and cell fusion experiments have so far shown existence of fourteen FA complementation groups and corresponding genes (FANCA, B, C, D1(BRCA2), D2, E, F, G, I, J(BACH1/BRIP1), L, M, N (PALB2), O(RAD51C), P (SLX4) and combined functionally with FA repair pathway (Deakyne et al. 2011; Rego et al., 2009; Stoepker et al., 2011). However, their exact role have not yet been completely elucidated. The FA-associated genes are not clustered, but dispersed throughout the genome. Next to BRCA2 (FANCD1) also BRCA1 was corroborated to be involved in FA and comprises another FA associated factor (Garcia-Higuera et al., 2001; Zdzienicka & Arwert, 2002). Thus, FA pathway is often called Fanconi/BRCA pathway.

Complementation group A (mutation in FANCA) stands for majority of the Fanconi anemia cases (over 66%), C in 5-15% and G in 5-15% in most of the populations. Only some ethnic groups have founder mutation which accounts for most cases of disease (e.g. in Ashkenazi Jews mutation IVS4+4 A→T allele). The prevalence of other complementation groups is rather rare and many mutations are reported in different Fanconi genes with various frequency (Buchwald, 1995; Deakyne et al., 2011; Joenje et al., 1997). The open-access database of Fanconi genes mutations is available in the internet (http://www.rockefeller.edu/fanconi/mutate).

The hypothesis over the defect in the repair of damaged DNA is central to the etiology of Fanconi anemia. Moreover, recent studies have shown that FA is also clinically related to other hereditary chromosomal instability syndromes and the proteins mutated in Bloom Syndrome, Nijmegen Breakage Syndrome (NBS), Ataxia Telangiectasia (ATM) and Seckel Syndrome (ATR), which are also implicated and crosslink with FA pathway (Andreasen, D’Andrea, & Taniguchi, 2004; Nakanishi et al., 2002; Taniguchi et al., 2002). Therefore, an emerging body of evidence indicate Fanconi/BRCA DNA repair pathway proteins to operate in multiple DNA damage pathways, whereas FA-associated proteins have alternative roles. Disruption of any of the genes may lead to arrest of the DNA repair processes and accumulation of cancer-proneness events, which finally leads to non-FA associated cancer occurrence. Thus, Fanconi pathway appears to play remarkable role in the DNA repair capacity of a cell.

5.1 FA and head and neck cancer

Numerous clinical data reports, that FA patients demonstrate a high incidence of aggressive forms of squamous cell carcinoma, especially at young age, predominantly in head and neck sites (HNSCC). The estimated risk is increased over 700 times higher compared to non-FA population and rises much above the cumulative risk effect of environmental factors for this cancer. Average age of HNSCC patients is usually above 60 years who drink and smoke, in
contrast to group so-called “young adults” of age below 40-45 years and not possessed by habits. Interestingly, FA patients are usually non-smokers and non-drinkers and demonstrate higher risk of HNSCCs with 40% cumulative incidence by the age of 40 years with 2-year survival rate much below 50% (Kutler et al., 2003). Seemingly, FA -associated squamous carcinoma of head and neck do not differ considerably from sporadic HNSCC, except hypersensitivity to crosslinking agents. FA/HNSCC patients are more vulnerable to the exposition of oral squamous mucosa as a first line absorbing carcinogens. Therefore, predominant site of cancer occur in oral cavity, oropharynx and tongue which is a major cause of high mortality in these patients. The repair of ICLs is mainly driven during cell arrest in late S/G2 phase. G2 phase is noticeable prolonged and, thus it rise the risk of viral infections, particularly Epstein–Barr virus (EBV) and oncogenic types of human papilloma virus (HPV). They integrate preferably to damaged sites of DNA. In previous study of FA/HNSCC over 80% have been infected with HPV (none of these showed mutation in TP53) in respect to 36% in non-FA HNSCC patients (Kutler et al., 2003). However, other studies could not demonstrate such high differences, but the disproportion was undisputed. Noteworthy is also that younger patients characterize with distinct sexual behavior, with higher preference to oral sex and thus high prediction of being HPV positive (Lustig et al., 1995; Rosenberg, Alter, & Ebell, 2008).

FA/HNSCC patients undergo strictly modified cancer therapy, eliminating ICLs inducers. Hematopoietic stem cell transplant (SCT) is nowadays the only efficient treatment for direct restoration of blood cells production in Fanconi anemia patients. There have been much of difficulties to optimize SCT protocols because of high toxicity of procedure to patients. From the other, side heavy SCT conditioning is frequently a cause of induction of squamous cell carcinomas, particularly head and neck cancers (Alter, 2005; Rosenberg et al., 2005).

It has been also suggested that single unrepaired ICLs in FA patients may be enough to promote the translocation of oncogenes or the deletion of tumour suppressor genes. Moreover, specific and well known oncogenic translocations such as loss of 1q, 3q or 9p and seems to be favored in FA (Sala-Trepat, et al., 1993; Tonnies et al., 2003).

5.2 DNA cross-linkers

As previously mentioned DNA in Fanconi anemia patients undergoes damage mostly by exposition to cross-linkers. Interstrand crosslinks (ICLs) are extremely toxic DNA damage type. Many drugs (mainly alkylating agents) producing interstrand cross-links in cellular DNA have been commonly used in clinical chemotherapy of solid and hematological malignancies. The idea of usage of crosslinkers to treat cancer was born out of horror of the Second World War. The autopsies of victims killed with chemical weapons (e.g. sulphur mustard) have shown selectively attacked blood white cells. The hypothesis has been established that those chemicals could have been useful for the treatment of leukemia (Deans & West, 2011) and further to other cancers. These include the nitrogen mustard class in use until now (e.g. melphalan, cyclophosphamide, chlorambucil etc), natural compounds: (mitomycin C, psoralen, aldehydes, formaldehydes), platinum drugs (cisplatin, carboplatin, oxaliplatin). Some physical agents such as UV light exposure or reactive oxygen species (ROS) are also capable to induce ICLs. Platinums and nitrogen mustard class require two active leaving groups, which are absorbed inside the cell by the sequential displacement of
chloride ions by H$_2$O molecules. This active form predominantly crosslink DNA at N$^7$-position of guanosine or adenosine on the opposite strands and forming ICLs. Mitomycin C and psoralens contain planar rings that must be activated by photon-mediated cycloaddition or cycloreduction, and attack DNA bases. The covalent bounds between two strands of DNA produced by cross-linkers leads to inhibition of fundamental processes such as replication and protein biosynthesis, preventing unwind for polymerase access. Clustering both DNA strands significantly reduces ability of DNA repair machinery to operate, and repair of cross-link involve a highly complexed networks. At the higher structural rank this cause high chromosomal instability (Bauer et al., 2008).

Noteworthy, major FA diagnostic method is still based on the cytogenetic analysis of lymphocytes that have been treated with ICL-inducing agents (MMC or DEB cross-linker) where chromosomal breaks are counted. As result, FA cells demonstrate highly increased levels of chromosome aberrations, such as: chromosome breakages and radical forms of chromosomes (Auerbach, 1988; Li et al., 1999).

5.3 DNA repair by FA/BRCA pathway

FA/BRCA pathway is currently conceived as the coordinator of several repair systems, including homologous recombination (HR), nucleotide excision repair (NER) and translesion synthesis (TLS). However, a major pathway for the repair of DNA interstrand cross-links (ICLs) is homologous recombination (also known as homology-directed repair, HDR). Postulated and partially elucidated model of FA processing with ICL repair begins when the replication forks are stalked and lesion recognition replication protein A (RPA) is recruited and responsible for stabilizing and coating of single DNA strands (L. C. Wang, Stone, Hoatlin, & Gautier, 2008). ICL invokes also FANCM with associated proteins (RMI 1 and RMI 2) which leads to the assemble of FA core complex (FANCA,B,C,E,F,G,L, M and N). FANCM also recruits the Bloom’s syndrome complex (BTR), and cooperates with RPA protein. This activates checkpoint ataxia telangiectasia kinase ATR and its downstream effector kinase CHK1 and Rad3-related (ATR)–CHK1 signalling cascade. ATR and CHK1 phosphorylate several components of the core complex in order to activate them. UBE2T, the ubiquitin-conjugating enzyme (E2) binds to FANCL, the ubiquitin ligase subunit of the core complex. Activate core complex monoubiquitinate FANCD2 and FANCI (ID complex) a key event in FA pathway. BRCA1 and BRCA2 complex initiates homologous recombination and stabilizes the stalled replication fork. Monoubiquitinated FANCD2 forms DNA damage inducible foci together with core complex and several other downstream FA-associated DNA repair proteins such as: structure-specific nucleases and translesion polymerases, MRN complex (MRE11, NBN, RAD50), BRIP1, PALB2, FANCO and further participate and cooperate in ICL removal (Fig.2). Homologous recombination is performed on the basis of homologous sequence on sister chromatids and the mechanism in itself is similar to crossing-over during meiosis (Deakyne et al., 2011.; Guervilly et al 2008; Machida et al., 2006; Rego et al., 2009; Takata et al., 2009).

5.4 Disruption of FA/BRCA pathway in HNSCC

The DNA damage response pathway controlled by the Fanconi anemia/BRCA pathway genes can be disrupted by either genetic or epigenetic events.
Most FA-associated genes have a wide variety of mutations in FA/HNSCC patients and include deletions, frameshifts, stop codons, splice-site mutations and missense mutations.

Fig. 2. Exemplary model of FA/BRCA pathway.

At this point of limited evidence, it seems that heterozygosity for any of FA gene mutation except BRCA2 generates higher susceptibility to cancer, whereas biallelic mutation in single FA genes contribute to earlier cancer development and generally poorer prognosis. However, no specific mutations have been associated with head and neck cancer. Some array-based data (Sparano et al., 2006) demonstrates the copy number alterations (CNA) in regions covering FA genes BRCA1, BRCA2, FANCD2, and FANCG but no direct associations with the disease were found. In contrast, FANCA has been analyzed in arrayCGH-based study (Bauer et al., 2008), where the region 16q23–q24 (FANCA locus) showed a significant gain associated with lower survival rates in HNSCC patients.

Epigenetic mechanisms such as DNA methylation, a main posttranscriptional DNA regulation plays a predominant role in majority of developmental processes and regulates expression of most genes. Moreover, it maintains integrity of chromatin through stabilizing its structure. DNA methylation level is usually measured by methyl groups attached to position 5 in pyrimidine ring of cytosines.

Somatic inactivation of the FA pathway by epigenetic silencing has been observed in several different types of sporadic cancer (Lyakhovich & Surralles, 2006), such as bladder cancer (Neveling et al., 2007), ovarian cancer (Lim et al., 2008), breast cancer (Wu, Shin-ya, & Brosh, 2008) cervical cancer (Narayan et al., 2004) and of course in HNSCC (Marsit et al., 2004;
Szaumkessel et al., 2011; Wreesmann et al., 2007). Our own study has demonstrated the disruption of DNA methylation of promoter region in FANCA, BRCA1 and BRCA2 in squamous laryngeal carcinoma cell lines and primary tumors (Szaumkessel et al., 2011). BRIP1 and FANCB have been shown to be higher and more frequently methylated compared to normal mucosa (Smith et al., 2010). FANCB is suggested to play important role in HNSCC pathogenesis as the hypermethylation was detected almost exclusively in cancerous samples. In other study (Wreesmann et al., 2007) FANCB, FANCF, FANCJ and FANCM were demonstrated to be affected by downregulation in HNSCC, however no methylation changes has been found comparing to non-tumor controls. FANCF promotoric DNA hypermethylation was demonstrated to be associated with smoking habit as the established risk factor in HNSCC (Marsit et al., 2004) but with no further reflection in other studies.

Recent years FA pathway is becoming of high interest because of versatile nature in respect to DNA repair. This would suggest that FA genes might be potentially widely used targets for future therapies of head and neck. Specific mutations or/and known deregulation mechanisms could provide a knowledge in order to design a targeted drugs to enhance existing anti-cancer therapies.

6. Summary

Despite the action upon improving the classical surgery and radio- and chemotherapy, a genetic background plays the supportive role and promise a significant enhancement in prevention and/or head and neck cancer curability. Last years has brought much of understanding in HNSCC biology, whereas many markers have been identified and utilized for genetic testing and therapies. Modern high-resolution techniques (e.g. CGH, aCGH, microarrays) has pointed relatively important DNA hotspots and greatly contributed to overall knowledge. However, the pathogenesis of HNSCC seems to be still elusive and ambiguous. The expectations always rise together with emerging new technologies and promise to develop brand new anti-cancer therapies.

7. References


Head and Neck Cancer provides an interesting and comprehensive overview of all aspects of head and neck cancer including overviews of the disease, basic science aspects pertaining to the disease, diagnosis, treatment and outcomes for patients with this disease. The chapters written by world renowned experts cover the entire discipline of head and neck oncology and include discussions of regional disparity is, advances in basic science understanding, advances in her radiotherapy, chemotherapy and targeted agents as well as a focus on reconstruction, prostheses, and aspects of quality of life and health outcomes. The book is designed to be both practical and comprehensive for every physician treating his complex disease.

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