

## Nucleotide excision repair and head and neck cancers

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### 1. ABSTRACT

Genome integrity maintenance is crucial for cell survival and for counteracting cancer onset and progression. Mammary cells invest great amount of energy in DNA repair, in order to avoid errors accumulation in DNA sequence. Nucleotide Excision Repair (NER) removes a broad spectrum of DNA damages, mainly bulky DNA lesions. Tissues of Head and Neck region are heavily exposed to bulky lesions inducing carcinogens, this making NER process of great interest in the field. Here we review the recent literature about NER in HNC and we also discuss the role played by NER in HNSCC in the chromatin context; to this aim we particularly focus on the role played by histones chaperon CAF-1, essential in restoring the chromatin structure following DNA replication and DNA damage repair, including NER. A better understanding of basic mechanisms underlying the DNA damage response, particularly involving NER, especially in the chromatin context, will provide us with new promising way to bypass the repair block, possibly becoming an unexpected mode of "transversal" control also of the proliferative deregulation, classically observed in HNSCC.

### 2. INTRODUCTION

For Head and Neck cancers we usually refer to tumors originating from nasal cavities and paranasal sinuses, pharynx (rinopharynx, oropharynx and ipopharynx), salivary glands, oral cavity and larynx. About 12,000 new cases of malignant head and neck cancers are diagnosed every year in Italy where these

tumors account for about 3% of all malignancies. The Italian incidence rate is 12 cases per 100,000 inhabitants, while in the whole Europe it is equal to 18 per 100,000 inhabitants. 90% of all tumors of Head and Neck region are squamous cell carcinomas (HNSCC: Head and Neck Squamous Cell Carcinoma); the remaining 10% are melanomas, lymphomas, sarcomas and tumor of other histology as salivary gland tumors. Men are usually more frequently affected than women (ratio being about 6:1) with an average age ranging between 50 and 70 years old (salivary gland tumors and sarcomas usually hit younger patients). Most affected sites for squamous cell carcinomas are ipopharynx, oropharynx, rinopharynx and oral cavity. Head and neck cancers are a very heterogeneous group of tumors, for etiopathogenesis, histology, natural history and mutational status. 75% of Head and Neck cancers are directly related to the association between tobacco smoke habit and alcohol consumption (1). HPV infection (mainly HPV16 and less frequently HPV18) (2-4) is at the basis of neoplastic transformation also in those cases where canonical risk factors do not apply. HPV related cancers are usually oropharynx squamous cell carcinomas of younger population, not preceded by clinically evident preneoplastic lesions, mainly linked to the sexual habits (number of partners, oral sex). In US more about 70% of oro-pharyngeal cancers are estimated to be HPV-positive and tend to increase. HPV positivity is a favorable prognostic factor in terms of tumor behavior and of response to chemo and radio-therapy: HPV positive tumors, in fact, are usually more sensitive to chemotherapies and to

radiation therapy. One of the possible explanations about the different behavior between the HPV positive and negative HNSCC rely on the different pathogenesis: HPV proteins E6/E7 epigenetically inhibits cell cycle proteins Rb and p53 (in HPV positive tumors), while in alcohol and tobacco associated cancers inactivation of the tumor suppressors occurs mainly by genetic mutations. Recently, a possible third class of HNSCC has been described with the coexistence of high risk HPV strains and a documented alcohol and tobacco exposure, envisaging a synergic effect between the two factors (5). The EBV virus has also been associated with HNSCC affecting young people.

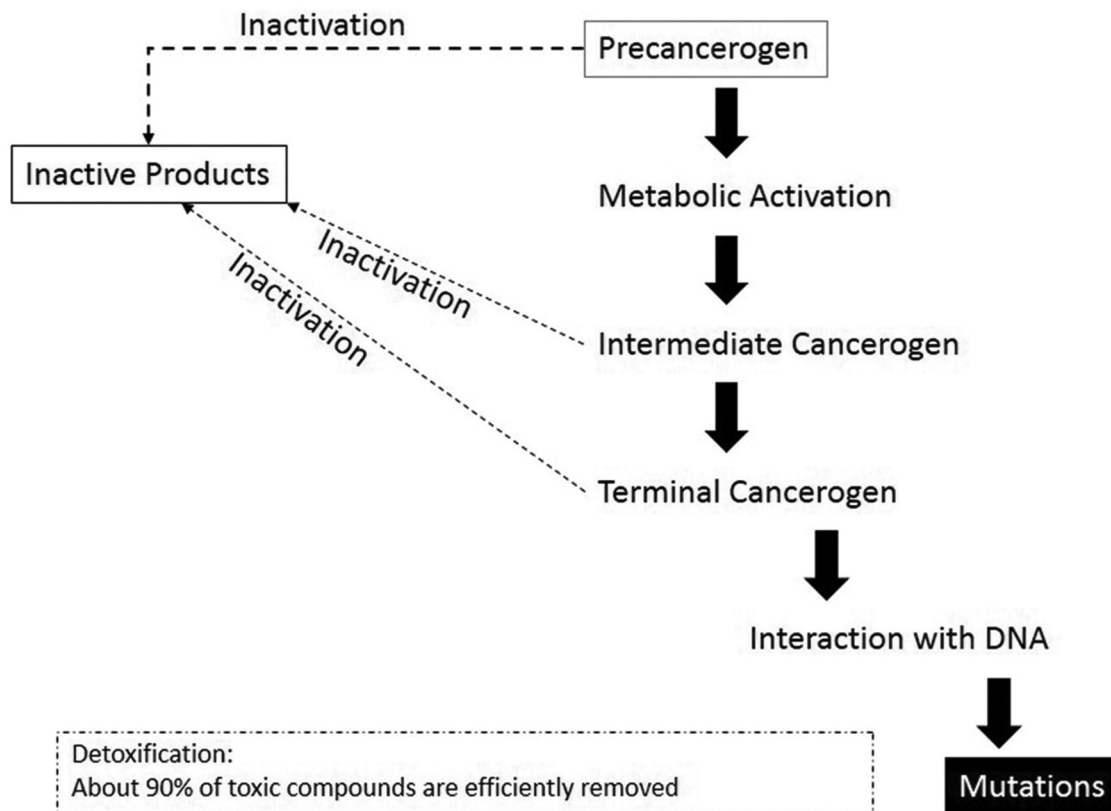
Fanconi anemia, a cancer-prone genetic disorder causing aplastic anemia, characterized by deficient DNA damage repair, has also been described to favor HNSCC. The mutation landscape of cancers of head and neck region reveals the extreme heterogeneity of these tumors (6). The molecular signature of these tumors show great variations depending whether the tumor is alcohol/tobacco associated or HPV positive; in fact, HPV positivity inversely associates to TP53 mutations, moreover alcohol and tobacco associated tumors usually show more mutations than the HPV positive counterpart (7). The most frequently mutated genes found in HNC are TP53, CDKN2A, PTEN, PIK3CA, HRAS and NOTCH1. It's interesting to note that in head and neck region cancers there is a prevalence of mutations in tumor suppressor genes, rather than oncogenes, this making much more difficult to find new "drug-able" molecular targets to personalize treatments (6,8-11). Staging of HNC commonly follows the UICC/AJCC guidelines (12). Tumors at initial stages are commonly treated with surgery or radiotherapy with similar results for some tumor sites (such as glottic larynx). Only exceptions are rinopharyngeal tumors whose treatment is mainly radiotherapy eventually associated to chemotherapy. Advanced stage tumors (stage III and IV) are mainly treated by surgery, especially oral cancers, although in last decades combined approaches with chemo and radiotherapy have been studied in order to improve the efficacy of radiotherapy. Several clinical studies have assessed the efficacy of chemotherapy and its use is highly recommended. The combined treatment with platinum based chemotherapy and radiotherapy has to be considered the standard treatment in locally advanced head and neck squamous cell carcinomas (III and IVA-B) (13). Chemotherapy has also to be considered the choice treatment in operated patients, with positive margins and/or excapsular nodal extension, and with good performance status (14-17). Several clinical studies have considered a platinum-based chemotherapy as neoadjuvant therapy before the locoregional surgical treatment. To date, the neoadjuvant chemotherapy has a defined role only to the aim of preserving the organs in case of ipopharyngeal-laryngeal tumors (18-22). Up to date we don't know many biomarkers predictive of therapy response. The viral etiology (HPV for oropharyngeal and

EBV for rinopharyngeal tumors) is a known prognostic and predictive marker (2-5,23). Recently mutational status of TP53 gene has been associated to prognosis in patients treated by surgery; prognosis is, in fact, affected by the presence and by the kind of mutation (24,25). EGFR hyperexpression has been shown to be a negative prognostic factor in terms of response to radiotherapy (26) as well as tobacco smoke and BMI, even in case of HPV positivity, this underlining the importance of life styles in modifying the therapy response (26). Overall, a better understanding of HNSCC tumor biology and the uncovering of more reliable prognostic and predictive biomarkers is needed in order to improve HNSCC patients response to therapy and quality of life. To this aim an interesting and actually debated field of investigation is the involvement of DNA damage response pathways in the pathogenesis, prognosis and response to therapy of HNSCC. As a matter of the facts, the DNA damage is strictly tied to cancer being cause of cancer, therapeutic strategy and responsible of many of side effects of current therapeutic strategies. The DNA damage response, on the other side, is an early anticancer barrier for many human solid tumors and mutations in DNA damage response pathways genes are often cancer prone.

### **3. GENOME STABILITY MAINTENANCE AS A CRITICAL DETERMINANT FOR CELL SURVIVAL AND NEOPLASTIC TRANSFORMATION**

Our genome is constantly under attack. Threats to the integrity of the genetic information derive from a multitude of causes coming both from outside (e.g. exposure to tobacco-smoke constituents, sunlight, dietary constituents) and inside the cell (e.g. free radicals associated with oxygen metabolism). Each cell of living organisms spend a large amount of energy in order to repair the DNA damages and failure to accomplish this important task might result in a variety of diseases. In particular, the maintenance of genome integrity is a big issue in cancer field (27). The negative effects that DNA damage is able to elicit on a single cell and, in a larger scale, on the entire organism, can be schematically divided in short-term effects and long-term ones. In the short term, damages to DNA might affect gene transcription and can elicit a DNA damage response; the signalling pathways that activate in response to DNA damage usually drive to cell cycle arrest, to allow damage to be repaired, or, whenever the damage is too extended to be repaired, to cell death by necrosis or by apoptosis or to cell senescence. In the long term, unrepaired or faulty repaired mismatches or DNA breaks can lead to an unfaithful DNA replication and the genetic alteration that follows is very often cause of neoplastic transformation (28).

As said, we recognise exogenous and endogenous sources of genotoxic stress: exposure to ionizing radiation, UV light and several chemicals, as well



**Figure 1.** Removal of toxic compounds.

as exposure to cellular metabolites that are constitutively produced in a living cell (such as oxygen free radicals), might causes DNA damage in the form of breaks or adducts. Cell survival and the maintenance of genome integrity, especially after the exposure to a genotoxic insult, rely mainly on the efficiency of DNA damage sensing and repair machineries. Those genes whose products are involved in i) detecting DNA damage and activating the repair machinery, ii) directly repairing damaged DNA, and iii) inactivating or intercepting mutagenic molecules before they have damaged the DNA (29) are called caretaker genes and they are acknowledged as tumour suppressors (30). Many cancers are characterized by caretaker genes loss during progression; this loss might be achieved via gene deletion, inactivating mutations or epigenetic repression. Although genomic DNA is by itself an unstable molecule (31), genome instability, meaning an extreme predisposition to accumulate mutations, is an hallmark of cancer (29). Cancer cells are, indeed, characterized by an increased rate of mutation (32).

#### 4. ENVIRONMENTAL CAUSES OF CANCER

By definition, “carcinogen” is considered any factor capable to favor or to cause the onset of cancer. Most of the chemicals carcinogens we know today are

genotoxic compounds. The genotoxic compounds alter, directly or indirectly (by their metabolic derivatives) the DNA molecules.(by adduction, substitution, base oxidation). Very few non-genotoxic cancerogens are known, whose are thought to induce epigenetic alteration of gene expression. The typical metabolic processing of a cancerogen is depicted in Figure 1. For example, the Benzo(a) pyrene, a classic DNA-damaging carcinogen in tobacco smoke and in the ambient environment, is biologically activated, *in vivo*, by cytochrome P450 and peroxidases, forming highly toxic electrophilic and free-radical reactive intermediates such as BPDE, that can irreversibly damage DNA by non-covalent intercalation and covalent bounding or oxidation (33,34). One pathway of BPDE covalent action leads to formation of covalent adduct primarily with the exocyclic amino-group of gGUO; the second pathway is a DNA-dependent hydrolysis of the diol-epoxide to tetrols (35-37). The determination of DNA adduct structures has been of critical importance to determine the repair mechanisms of BPDE-dependent DNA lesions, for what NER proved to be the pathway of choice (38).

DNA lesions are immediately followed by DNA repair (39). The choice of a specific DNA repair pathway usually depends on the type of DNA damage;

the relationship between lesion type and mechanism of repair could be summarized as follows: mismatches or structural abnormalities at the replication forks are repaired by the mismatch repair pathway; DSBs are repaired by homologous recombination; damaged bases are repaired by BER; NER occurs in case of bulky DNA lesions which are exclusively repaired by nucleotide excision repair (40). The majority of physical and chemical carcinogens (except for ionizing radiations and most alkylating agents) produce bulky lesions. The vast majority of cancers of head and neck region (nasal cavity, sinuses, lips, mouth, salivary glands, throat, or larynx) are squamous cell carcinomas, beginning in the squamous cells that line the moist surfaces inside the head and neck. Tobacco use (including “passive smoke”), together with alcohol consumption and HPV infection, is an important risk factor for head and neck cancers, with a particular predilection of oral cavity, oropharynx, hypopharynx and larynx districts (41,42). Head and neck cancers account for approximately 3 percent of all cancers in the United States, where it has been estimated that about 52,000 individuals of both sexes have been diagnosed of HNC in 2012 (43). Epidemiological studies have correlated tobacco smoking habit with less efficient DNA repair (44-46) indeed it has been shown also experimentally that a less efficient DNA repair in circulating lymphocytes correlates with an increased risk of developing HNC, in a dose-dependent manner (47). Lymphoblastoid cell lines obtained from HNC patients had minor alterations in DNA repair function, however the mutagen sensitivity correlated with NER capacity (48). Correlation between reduced NER associated DRC (DNA repair capacity) and increased risk to develop cancer has been reviewed elsewhere (40).

## 5. NUCLEOTIDE EXCISION REPAIR (NER)

Mismatches between the strands of DNA are among the major targets for repair systems and are usually corrected by excision repair. Base excision repair (BER) systems directly remove the damaged base and replace it in DNA, while nucleotide excision repair (NER) systems excise a sequence that includes the damaged base or bases replacing it with a stretch of newly synthesized DNA. As a general consideration, we might say that most cells possess four different categories of DNA repair system: Direct, Excision, Mismatch and Recombination repair systems. The “Direct” repair systems act directly on damaged nucleotides, converting each one back to its original structure. The “Excision” repair involves excision of a segment of the polynucleotide containing a damaged site, followed by resynthesis of the correct nucleotide sequence by a DNA polymerase. The “Mismatch” repair corrects errors of replication, again excising a stretch of single-stranded DNA containing the mutated nucleotide and then repairing the resulting gap. Finally, the “Recombination” repair is used to correct double-strand breaks (49).

Nucleotide excision repair removes a broad spectrum of DNA damages excising and resynthesizing a region of a polynucleotide (50,51). The NER process differs from base excision repair because it is not preceded by selective base removal and a longer stretch of polynucleotide is excised; showing, therefore, a much broader specificity. Chromatin rearrangements occur during NER; the first evidence came from the *in vivo* observation that the nuclease accessibility of DNA is modulated during UV-induced DNA synthesis in mammalian cells (52,53). We recognize two types of NER: the transcription coupled nucleotide excision repair (TC-NER), and the global genome nucleotide excision repair (GG-NER). TC-NER is strictly associated to transcription and its activation involves the RNA polymerase II while GG-NER activation is unrelated to transcription. The repair via nucleotide excision of the transcribed strand of double helix is generally much faster than untranscribed regions of the genomes maybe because in the first one act both the TC-NER and the GG-NER (51).

At the molecular level, we could summarize the core events of NER mechanism as follows (54): (I) Whenever a DNA lesion, distorting the double helix, occurs, XPC-hHR23B senses the distortion in GG-NER leading to conformational alterations of the DNA. In transcription-coupled repair (TC-NER) lesions are detected by elongating RNA Pol II blocked by, e.g., CPDs. (II) Once XPC-hHR23B binds to distorted helix, it attracts at lesion TFIIH (and possibly XPG). TFIIH creates a 10- to 20-nucleotide opened DNA complex around the lesion by virtue of its helicases XPB and XPD; this step requires ATP. Once the helix has been opened by helicases, XPC-hHR23B may be released at this or one of the subsequent stages. In TC-NER CSA, CSB, TFIIH, XPG, and possibly other cofactors displace the stalled Pol II from the lesion, which now becomes accessible for further repair processing. (III) XPA and RPA stabilize the 10- to 20-nucleotide opening, create by TFIIH, and drive the position of other factors. XPA binds to the damaged nucleotides, RPA to the undamaged DNA strand. The RPA stretching formation plays an important role in full open complex formation stabilized by XPG. (IV) XPG, positioned by TFIIH and RPA, makes the 3' incision, while ERCC1-XPF, positioned by RPA and XPA, makes the second incision 5' of the lesion. (V) Finally, the dual incision is followed by gap-filling DNA synthesis and ligation.

For a comprehensive list of NER involved genes please refer to Table 1. The actual knowledge about the NER process is based on both *in vitro* and *in vivo* experiments; the first ones take advantages of the ability of yeast, xenopus, drosophila or eukaryotic cell free extracts to recapitulate *in vitro* all the aspects of DNA damage repair process by using as DNA source typically a molecule of naked DNA previously exposed to



**Table 1.** Human NER associated genes as reviewed in (67). Nucleotide excision repair (NER) associated genes

Nucleotide excision repair (NER) associated genes (XP = xeroderma pigmentosum)	Gene associated function	Locus
XPC	Binds DNA distortions	3p25.1.
RAD23B	XPC, RAD23B, CETN2	9q31.2.
CETN2		Xq28
RAD23A	Substitutes for RAD23B	19p13.1.3.
XPA	Binds damaged DNA in preincision complex	9q22.3.3.
DDB1	Complex defective in XP group E DDB1, DDB2	11q12.2.
DDB2 (XPE)		11p11.2.
RPA1	Binds DNA in preincision complex RPA1, RPA2, RPA3	17p13.3.
RPA2		1p35.3.
RPA3		7p21.3.
TFIIH	Catalyzes unwinding in preincision complex	
ERCC3 (XPB)	3' to 5' DNA helicase	2q14.3.
ERCC2 (XPD)	5' to 3' DNA helicase	19q13.3.2.
GTF2H1	Core TFIIH subunit p62	11p15.1.
GTF2H2	Core TFIIH subunit p44	5q13.2.
GTF2H3	Core TFIIH subunit p34	12q24.3.1.
GTF2H4	Core TFIIH subunit p52	6p21.3.3.
GTF2H5 (TTDA)	Core TFIIH subunit p8	6p25.3.
CDK7	Kinase subunits of TFIIH CDK7, CCNH, MNAT17. <b>Chromatin dynamics at DNA replication and DNA damage sites.</b>	5q13.2.
CCNH		5q14.3.
MNAT1		14q23.1.
ERCC5 (XPG)	3' incision	13q33.1.
ERCC1	5' incision DNA binding subunit	19q13.3.2.
ERCC4 (XPF)	5' incision catalytic subunit	16p13.1.2.
LIG1	DNA ligase	19q13.3.2.
NER-related		
ERCC8 (CSA)	Cockayne syndrome and UV-Sensitive Syndrome; Needed for transcription-coupled NER ERCC8, ERCC6, UV-sensitive syndrome	5q12.1.
ERCC6 (CSB)		10q11.2.3.
UVSSA (KIAA1530)		4p16.3.
XAB2 (HCNP)	XAB2	19p13.2.
MMS19	Iron-sulfur cluster loading and transport	10q24.1.

DNA damage agents (e.g. UVC light) (55-58). Although this approach has unveiled many of the molecular details of NER apparatus and function, it is of great interest to consider the NER process within the chromatin context, a condition closer to what we may expect to happen within a living cell; the *in vivo* experiments, carried out mainly in cultured cells, have provided most informations to this point (59-61). An insight on how GG-NER works in the context of chromatin has been recently provided by Yu *et al.* showing how GG-NER drives UV-induced chromatin remodelling by controlling histone H3 acetylation levels in chromatin (62). The hierarchical activation of NER machinery proteins follows the so-called ARR model, a complex network of chromatin modifying, remodelling, assembly factors, signalling pathways and repair proteins, postulated by Smerdon in 1991 (63,64). ARR stands for Activation, Repair and Restore; this model recognises, in fact, three consecutive steps in the repair process: sensing the damage with subsequent activation of the machinery, the repair process itself and a recovery step during which chromatin is repacked and recondensed to the original state (59,63,65,66). General ARR model involves Chromatin remodelling/Modification factors (that allow the repair factors the access to chromatin), the Repair machinery (responsible for the repair itself), and the Chromatin assembly factors (responsible for the *restitutio ad integrum* of the chromatin).

## 6. EVIDENCES OF NER INVOLVEMENT IN HNC

The risk of squamous cell carcinoma of Head and Neck has been associated with poor DNA repair phenotype in response to benzo(a) pyrene diol epoxide, a carcinogen released by tobacco smoke (47,68-70). The efficiency of NER DNA repair capacity is significantly affected by polymorphisms in NER genes (71). During last decade, several published research article reported a significant correlation between polymorphisms of NER genes and increased risk of HNSCC (72-90); fewer reports can be found about relationship between NER genes polymorphisms and progression of H&N cancer (88), outcome of advanced-stage tumors (91), response to radiotherapy (especially in combination with RAD 51 polymorphisms) (92) and to cisplatin-based chemotherapy (93), progression of the disease (94). Genetically determined NER DNA repair capacity may modulate not only cancer risk, but also prognosis: an increased risk of second primary malignancy in patients with SCCHN has been associated to NER genes SNP (95), as well as susceptibility to recurrence of HNSCC (96). Sometimes, a single polymorphic allele proved not to be associated with an increased risk of HNSCC, on the contrary a combination of alleles, such as having both ERCC1 809CC and ERCC2/XPD 23591A alleles, statistically associates to increased risk of SCCHN (81). Association with NER genes SNPs, cigarette smoking and risk of Head and Neck cancer has also been evaluated (97-99). Expression studies have also

been performed in order to correlate abundance of NER proteins to survival in HNSCC (100), and as a marker of susceptibility to HNSCC (101). An increased expression of NER proteins has been found in metastases of SCCHN, probably contributing to resistance to cisplatin-based chemotherapy (102). A significant association has been found between risk of oral premalignant lesions and SNPs of several NER genes, this further confirming the strict relationship between impairment of NER process and development of HNSCC (103).

## 7. CHROMATIN DYNAMICS AT DNA REPLICATION AND DNA DAMAGE SITES

Great attention has been paid on the role of chromatin dynamics as critical determinants in many nuclear events and in pathological conditions such as tumor development and progression (104,105). Whichever is the metabolic process involving the DNA molecule, it should always be considered in the chromatin context. Within the cell, the nuclear DNA is tightly packaged in the chromatin structure. The nucleosome is the fundamental unit of chromatin and it is composed of an octamer of the four core histones (H3, H4, H2A, H2B), around which 147 base pairs of DNA are wrapped. DNA packaging follows several orders of wrapping and is fundamental for the maintenance of the genome stability regulating the DNA-based activities as DNA replication, transcription and repair. Chromatin structure poses structural constraints likely to challenge vital processes like DNA replication and repair (106); detection and repair of DNA lesions, as much as recognition and activation of replication origins and progression of replication forks, are in fact some of the processes than more than others have to cope with the several orders of chromatin packaging (106,107). The relationship between the nucleosomes and the DNA damage repair process is controversial: first (*in vitro*) studies showed, in fact, an inhibitory effect of nucleosomes on the repair process (108) while *in vivo* studies seem to show the opposite (109,110), thus confirming the importance of a chromatin remodeling activity.

A current challenge is to understand how to integrate chromatin structure within the scheme of DNA repair and how it is associated with maintenance (or loss) of epigenetic information (64). Together with the genome instability, in fact, every living cells has to cope also with the epigenomic instability, whose consequences are not less dramatic than the first one. A major unresolved issue related to histone dynamics within damaged chromatin is whether preexisting nucleosomal histones are replaced by new histones within damaged chromatin or if they are recycled. It remains unknown how restoration of chromatin structure is achieved *in vivo*. Histone chaperones plays a critical role in maintaining and regulating chromatin structure driving histones deposition. Among the histone chaperones, the best known is CAF-1 (Chromatin

Assembly Factor-1) a heterotrimeric complex, formed by p48, p60 and p150 subunits, that plays a pivotal role in the epigenetic regulation of chromatin assembly and participates in the DNA damage repair, too. During the S phase of the eukaryotic cell cycle, the newly replicated DNA is rapidly assembled into chromatin. CAF-1 mediates the deposition of newly synthesised histones H3 and H4 onto nascent DNA and their assembly into nucleosomes, by PCNA association (111). CAF-1 was first described as a chromatin protein for which a DNA repair role was subsequently discovered (112,113); it is required for nucleosome assembly coupled to DNA repair and experiments in yeasts have shown that yeast mutants are UV sensitive (114,115).

Another histone chaperone involved in chromatin assembly is Asf-1 that has been described to synergize with CAF-1 following DNA replication or NER (116).

## 8. CAF-1 AND NER

Most of our knowledge about NER system is about activation and repair, less is known about the restore step. Recovery from Nucleotide excision repair mechanism involves several chromatin modifiers and, among them, the most studied is CAF-1. Of particular interest is to consider the role of CAF-1 during the NER, emphasizing the recovery process since CAF-1 drives histones repositioning after repair. CAF-1 complex, initially described as a replication-dependent chromatin assembly factor (117,118), has been widely associated to UV-repair by NER. Studies *in vitro* have shown that the complex is required for the assembly of nucleosomes around a repair site in a PCNA-dependent manner (65,113), CAF-1 and PCNA are, in fact, recruited to chromatin in UV-damaged cells (119); in particular, a phosphorylated form of CAF-1 is recruited to chromatin following UV exposure, as demonstrated in cell cultures (119). *S. Cerevisiae* lacking CAF, are sensitive to UV light (114,115). In order to drive chromatin assembly, CAF-1 complex binds post-translational modified histones H3.1. and H4, in particular has been shown that CAF-1 is associated with acetylated forms of histones H3.1. and H4 (117,120). A proficient CAF-1 function and NER are required for a stable *de novo* incorporation of histone H3.1. at sites of UV damage. Following deposition, histones H3 and H4 are rapidly deacetylated and although CAF-1 has never been demonstrated to act as a deacetylase, the p48 subunit of CAF-1 has been shown to associate with deacetylated activity (118). Several histones post-translational modifications identify sites of DNA within the chromatin, among them are ubiquitylated H2A foci (121). CAF-1 localizes to gH2AX sites and knockdown of CAF-1 p60 abolished CAF-1 as well gH2AX foci formation. Moreover, CAF-1 p150 was found to associate with NER factors TFIIR, 1q23, RPAp70, PCNA in chromatin. Successful NER of genomic lesions

and prompt CAF-1 mediated chromatin restoration link gH2AX incorporation to the sites of damage repair within chromatin (122). In *S. Cerevisiae* Rad53 controls the degradation of excess histones, in order to avoid their accumulation in cells (123); in mammalian cells, the CUL4-DDB-ROC1 ubiquitin ligase recently was found to mediate UV-induced H3 and H4 ubiquitination and facilitate nucleosome eviction (124).

## 9. CAF1 AND CANCERS OF HEAD AND NECK

CAF-1 involvement in several human cancers has been well documented. A large body of literature demonstrates that CAF-1 overexpression correlates with higher aggressiveness and poor prognosis. The nuclear expression of CAF-1 p60 is particularly increased in multiple types of cancer, proportionally to their adverse clinical behavior (125-129). Expression of CAF-p60 and p150 subunits, evaluated by IHC, has been found increased in tongue SCC (125) and salivary gland tumors (129); moreover, the over-expression of CAF-1 p60 subunit, together with cancer stem cell marker expression (eg Nestin) predicts the metastasizing behavior of oral cancer (130). CAF-1 subunits IHC expression in head and neck cancers has been conveniently investigated also by TMA technique (130).

The peculiar function of Chromatin assembly factors make them ideal candidates for a new therapeutic approach to treat malignant neoplasia; in particular, CAF-1 p60 has recently emerged as a promising target, inhibition of which could lead to cell death in aggressive tumors (125,127,129). Epigenetic alterations, especially the histone modifications, influence cellular metabolism and mainly affect the chromatin structure. The epigenetic inheritance includes DNA methylation, RNA-mediated silencing and histone modifications, although DNA methylation and histone acetylation are among the most frequent epigenetic modifications observed both in normal and neoplastic cells, the disruption of any of these three distinct and mutually reinforcing epigenetic mechanisms leads to an inappropriate gene expression, resulting in cancer development and other 'epigenetic diseases' (131,132). Chromatin assembly factors, such as CAF-1, are crucial for cell survival and to preserve the integrity of the genome. In the present review we focused mainly on the role of CAF-1 in the NER process in the chromatin environment; we specifically focused on the restore process, that in the hierarchical ordered events of the NER repair process, is the only chance the cell has to recover from the cell cycle checkpoints activation and reload the normal cell cycle machinery, following the restitutio ad integrum of the nuclear chromatin. Failing to do that means dramatic consequences to the cell fate. A better understanding of basic mechanisms underlying the DNA damage response, particularly involving nucleotide excision repair, especially in the chromatin context, will provide us with new promising way to bypass

the repair block, possibly becoming an unexpected mode of "transversal" control also of the proliferative dysregulation, classically observed in HNSCC.

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