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DNA Damage and Repair in Cancer Therapy

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Introduction

Most cancer therapies have their main mechanism of action DNA damage. Tumor cells have several ways to respond to this DNA damage: removal of the damaged area and reconstruction of the strand of DNA, activation of DNA damage checkpoint, changes in transcription profile of benefit to the cell and apoptosis in the case in which the DNA is seriously damaged [1,2].

We summarize in this article the most important cellular responses to DNA damage caused by chemotherapy.

DNA Damage

DNA damage includes both covalent and non covalent changes in the structure of DNA, like as base-pair mismatches, loops and bubbles arising from a string of mismatches.

We will see now the way in which the major chemotherapeutic agents induced the damage in the DNA:

Alkylating agents (eg. cyclophosphamide, nitrosoureas)

- Add methyl groups or alkyl groups to guanine.

- DNA strand cross-links (cross-links in DNA chain).

Antibiotics (eg. bleomycin, dactinomycin, doxorubicin)

- Binds to DNA, inhibits DNA replication and transcription.

- DNA strand breaks.

Inhibitors of topoisomerase I and II (eg. irinotecan, etoposide)

- Topoisomerase I causes a transient break in one strand of DNA and topoisomerase II causes breaks in the double helix, both of which guarantee the posterior process of DNA replication.

- Inhibition of this mechanism results in accumulation and stability of the topoisomerase-DNA covalent complexes and subsequently cell death.

Spindle poisons (eg. paclitaxel, docetaxel)

- Disrupts microtubule function.

Platinum

- Intra-strand, inter-strand DNA cross-links.

- In addition to direct damage to DNA, platinum is bound to proteins, altering the transmembrane transport of essential amino acids, the role of calcium channels, the mitochondrial respiration and transport of phosphate.

Antimetabolites (eg. methotrexate, pemetrexed, fluoropyrimidine)

- They block cell growth by interfering with DNA synthesis in S phase of cell cycle.

- Inhibit enzymes needed in the synthesis of nucleic acids behave as antagonists.

Tubulin inhibitors (eg. vincristine, vinorelbine)

- They block mitosis in metaphase. Specifically they bind to tubulin, inhibiting microtubule assembly and, consequently, the cell is in metaphase. The chromosomes are dispersed in the cytoplasm leading to cell death.

DNA Damage Recognition

There are three main mechanisms responsible for the recognition of DNA damage [3]:

1. Direct damage Recognition: it is based on the complementarity between a particular DNA damage and cognate protein (protein related individuals).

2. Multistep damage Recognition: matchmakers and combinatorial molecular recognition.

3. Recognition of DNA repair intermediates: DNA excision repair pathways damages involve removal of DNA nucleotides and replacement with newly synthesized DNA.

Cellular Response to DNA Damage

Depending on the attack in DNA, the cell can try to repair or induce apoptosis.

The main cellular responses to DNA damage are (Table 1):

Base excision repair (BER)

This system removes damaged base from DNA repair or DNA single strand breaks [4]. Usually non-bulky lesions are repaired by this system, those that do not distort the double helix structure of DNA. The pathway of excision repair base is the principal mechanism for repairing DNA alkylation and oxidative damage. Most of these injuries come from endogenous metabolism.

At first, a DNA glycosylase is activated after recognizing oxidized / reduced bases, methylated bases, deaminated or bases mismatches. This DNA glycosylase breaks the bond between the glycosyl group and the deoxyribose-phosphate, backbone of DNA. Later apurinic

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Base Excision Repair (BER)	*PARP1, PARP2, NEIL and XRCC1*
Nucleotide Excision Repair (NER)	RPA, XPA, XPC, TFIIH, XPG and XPF-ERCC1
Alternative Excision Repair (AER)	
Coupled Nucleotide Excision Repair Transcription (TC-NER)	ERCC6
Mismatch Repair (MMR)	MSH2 and MSH3
Translesion synthesis (TLS)	DNA polymerase kappa
Nonhomologous End Joining (NHEJ)	XRCC4 XRCC5
Homologous recombinational Repair (HRR)	BRCA1, BRCA2 and RAD 51
Cell Cycle Checkpoint Control	*p53*
Apoptosis	Caspases and Bcl-2 family

Table 1: The main cellular responses to DNA damage and the principle proteins associated.

or apyrimidinic site is recognized by an endonuclease that produces a nick at site 5' DNA. Then, a short-lyase cuts the 3' apurinic site. The deoxyribose-phosphate residue is removed by an exonuclease. Finally, the single nucleotide gap is filled in by repair synthesis by DNA polymerase.

Some of the genes involved in this system are *PARP1, PARP2*, *NEIL* and *XRCC1*.

The BER mechanism is the key to maintaining the integrity of cellular DNA. One reason is because it is the predominant repair pathway for the processing of small lesions bases. It is estimated that about 10,000 base lesions per mammalian cell per day, and hence its importance. Furthermore, deletion of genes in the population BER is lethal to the embryo.

PARP-1 (Poly-ADP-ribose polymerase) is an enzyme that produces large branched chains of poly-ADP-ribose [5]. This protein senses and binds to DNA nicks and breaks, resulting in activation of catalytic activity causing poly-ADP-ribosylation of PARP-1 itself. This produces a recruitment of other components of DNA repair pathways.

When *PARP1*, the most abundant member of the *PARP* family is inhibited, double-strand DNA breaks accumulate and under normal conditions are repaired via the BRCA pathway-dependent homologous recombination mechanism. It was thought that inhibition of *PARP,* in combination with DNA-damaging chemotherapeutics, would render tumors lacking BRCA function exquisitely sensitive, a hypothesis that has borne out in both preclinical and clinical studies. Given the shared clinicopathologic characteristics between BRCA-mutated and triple-negative breast cancers, the efficacy and safety of PARP inhibition is being tested in both settings [6]. There are several *PARP* inhibitors in clinical development, such as iniparib, olaparib and velparib.

A phase I clinical trial with olaparib was published in 2009. 60 patients were enrolled and treated. 22 were carriers of *BRCA1* or *BRCA2* mutations and only some of these patients had any antitumor response. No objective antitumor responses were observed in patients without known BRCA mutations [7].

From this study, we know that not all *BRCA1* or *BRCA2* carriers had a response to olaparib. Various *BRCA1* or *BRCA2* mutations may have resulted in differing homologous-recombination defects and sensitivities to PARP inhibition, or maybe differences in response could also have resulted from pre-existing genetic resistance.

As we have said before, triple-negative breast cancers have inherent defects in DNA repair. Another *PARP* inhibitor, iniparib, was tested in triple-negative breast cancer patients in a phase 2 study, comparing the efficacy and safety of gemcitabine and carboplatin with or without iniparib. In the iniparib arm the rate of clinical benefit was 56% vs. 34% (p=0.01) and the rate of overall response was 52% vs. 32% (p=0.02). The addition of iniparib also prolonged the median progression-free survival from 3.6 months to 5.9 months (HR 0.59; P=0.01) and the median overall survival from 7.7 months to 12.3 months (HR 0.57; P=0.01) [8].

XRCC1 interacts with most of the core components, mostly with DNA ligase III, polymerase beta and *PARP1*. It may play a role in DNA processing during meiogenesis and recombination in germ cells. Several studies have been published talking about polymorphisms in *XRCC1* in patients treated with cisplatin, finding controversy in the results [9-11].

Although we believe that the BER pathway has a very important role in preserving the integrity of cellular DNA, we think that more studies are needed to verify the real clinical impact of the PARP inhibitors and to identify patients who are really likely to benefit from this treatment.

Nucleotide excision repair (NER)

This process recognized and mends DNA primarily with bulky, helix-distorting damage potentially capable of blocking DNA replication or transcription [12].

Damaged nucleotides are removed by the "excision nuclease", a multisubunit enzyme system that makes dual incisions bracketing the lesion in the damaged strand. Between 24 and 32 nucleotides are usually removed. Subsequently, synthesis is repaired to fill the resulting vacuum and it is linked to the above. In humans, the NER system is formed by six repair factors, RPA, XPA, XPC, TFIIH, XPG and XPF-ERCC1 [10]. Some of them were described by mutations in genetic syndroms such as xeroderma pigmentosa (XPA) and Cockayne syndrome (CFS).

This is the most important repair system to process bulky lesions involving DNA distortion of the DNA strand. These lesions are formed by exposure to radiation or chemicals such as polycyclic aromatic hydrocarbons and DNA intercalating agents such as cisplatin, or protein adducts with DNA. The importance of NER is still emphasized by the fact that there are at least five genetic syndromes directly related to defects in this repair mechanism (xeroderma pigmentosum, Cockayne syndrome, trichothiodystrophy syndrome oculo-cranio-facial-skeletal).

XP genes are involved in the recognition and repair of lesions in global genome NER, while *CS* genes play a specific role in transcription coupled repair [13].

ERCC1 is an endonuclease involved in excision of the lesion; high tumor tissue levels of *ERCC1* mRNA in ovarian and gastric cancer have been associated with cisplatin resistance [14,15].

In a similar way, inhibition of *ERCC1* expression has been associated with reduced host cell reactivation of cisplatin-treated cells and increased cisplatin sensitivity [16,17].

The *ERCC1* expression was analysed in the International Adjuvant Lung Cancer Trial, demonstrating that there was a deficiency in 56% of the patients and was correlated with a significant improvement in survival following cisplatin-based chemotherapy; no benefit was observed in those patients with *ERCC1* expression maintained [18].

In 2002 an article was published about *ERCC1* mRNA expression in pretreatment tumor specimens from stage IV NSCLC patients treated with cisplatin based chemotherapy. Patients with low levels of *ERCC1* lived 15 months in comparison with the 5 months of survival of patients with high levels. The response rate was higher in tumors with low levels of *ERCC1* as well (52 vs. 36%), although this difference was not significant (p=0.38) [19].

Based on this promising clinical data, Rosell et al. published in 2007 a phase III clinical trial of customized chemotherapy according to ERCC1 mRNA levels in patients with stage IV NSCLC. Patients were randomized in a control arm to receive cisplatin-docetaxel and in a genotypic arm to receive cisplatin-docetaxel if they had low *ERCC1* mRNA levels or gemcitabine plus docetaxel it they had high *ERCC1* mRNA levels. Objective response was attained by 39.3% in the control arm and 50.7% in the genotypic arm (P =0.02) [20].

Alternative excision repair (AER)

This is a variant of the previous two. In this case the endonuclease V produces a cut in only one of the damaged sites, for example, in the 3'. This system has not been well described in humans.

Coupled nucleotide excision repair transcription (TC-NER)

This is a specialized form of NER and acts when the DNA damage occurs in regions that act as a template strand for transcription [21]. When RNA polymerase is stopped because of injury, this system moves beyond the damaged area and recruit repair factors, the same system as NER. Typically, 30 pairs of oligonucleotides are removed. The ERCC6 is one of the genes involved in this system.

The ERCC6 protein is a DNA-binding protein which has ATP-stimulated ATPase activity; there are contradictory publications reporting presence or absence of helicase activity. The protein appears to interact with several transcription and excision repair proteins, and may promote complex formation at repair sites. Single-nucleotide polymorphisms have been associated with overall survival in lung cancer [22].

Mismatch repair (MMR)

It recognizes and repairs base mismatches based and loops that form during DNA replication in mismatched nucleotides (but normal, i.e. not damaged). It often repairs those damages caused by oxygen radicals and alkylating agents. The proteins of this system may interact with other replication mechanisms such as BER or NER, suggesting a coordination of the entire process. Examples of these complex proteins are *MSH2* and *MSH3*.

A complex formed by *MSH2* and *MSH6* recognizes mismatched based, recruiting *MLH1* and *PMS2*, and then the repair is initiated (excision, DNA synthesis and ligation).

MMR functions as a guardian of the genome, repairing the mismatch without damage occurring during DNA replication. Although the inactivation of MMR proteins will not lead to cell death or other serious consequences, it greatly increases the rate of mutations throughout the genome due to unrepaired replication errors; this could initiate or promote carcinogenesis and confer a selective growth advantage in cells without MMR.

Defective in mismatch repair is known as microsatellite instability (MSI); a majority of cancers derived from patients with hereditary non-polyposic colorectal cancer (HNPCC) presents mutations in mismatch repair. Nearly of 85% of them have mutations in *MLH1* or *MSH2*. A 15% of sporadic colon cancer is also related with mutations in mismatch repair.

Many but not all tumors that contain MMR mutations can be identified by the presence of a high degree of MSI (MSI-H). The majority of patients with HNPCC have MSI-H tumors. Most laboratories use a panel of several microsatellite loci when testing for MSI. A panel consisting of three dinucleotide repeats and two mononucleotide repeats have been proposed as a standard test for MSI; a tumor is called MSI-H when at least two (40%) are affected by instability. While almost all MSI-H tumors are MMR deficient, most MSI-L tumors have no MMR defect.

The MSI-L phenotype has been associated with methylation of the promoter for the DNA repair gene 0-6methylguanine DNA methyltransferase (MGMT). It has been hypothesized that loss of expression of MGMT results in accumulation of methyl G:T mismatches and excess stress on the mismatch repair system, which ultimately leads to MSI-L. Being rare in HNPCC, MGMT methylation of loss or MGMT expression occurs in up to 25% of sessile serrated adenomas, 78% of dysplastic serrated adenomas, and 50% of serrated adenocarcinomas. These findings suggest that the factors leading to the MSI-l phenotype may be important in the serrated neoplasia pathway.

As we have said before, mutations and allelic loss of one of the MMR genes are responsible for the MSI phenotype in most cases of HNPCC. However, hypermethylation of the promoter region of some MMR genes and/or DNA hypomethylation with loss of imprinting is thought to underlie cases of sporadic CRC that display the MSI phenotype.

DNA hypermethylation specifically targets CpG dinucleotides, which are present in the promoters of many genes (including the MMRgene hMLH1). Although the stimulus that drives hypermethylation remains unknown, methylated CpG is bound by a family of proteins known as methyl-CpG binding domain proteins. These proteins form a multiprotein complex that alters chromatin conformation and silences gene expression.

A potential resistance to 5-fluorouracil chemotherapy has been described with mutations in mismatch repair.

The immunohistochemical expression of *MSH2* has also been studied as a predictive factor in patients with lung cancer treated with surgery and platinum-based adjuvant chemotherapy. In a sub analysis of the International Adjuvant Lung Cancer Trial it was determined the expression of *MSH2* in 673 patients. MSH levels were low in 38% of cases and high in 62%, there was a tendency to prolong overall survival in patients treated with chemotherapy and low *MSH2* expression (p <0.03), but not when your expression was high. Furthermore, when combined the analysis taking into account the expression of *MSH2* and *ERCC1*, the benefit of chemotherapy was lower as they expressed greater amounts of these markers. Chemotherapy prolongs overall survival in the subgroup of patients with low *MSH2* / low *ERCC1* (HR, 0.65, 95% CI, 0.47 to 0.91, P = 0.01 [23].

Translesion synthesis (TLS)

It is a damage tolerance mechanism, allowing replication to continue despite the damage by a DNA polymerase or more than one. The correct base is inserted opposite the lesion during the formation of the daughter strand, belongs to the complex POLI POLK.

DNA polymerase kappa (POLkappa) is frequently overexpressed in human lung cancer tissues. It is also described as an association between elevated expression and p53 inactivation in lung cancer tissues.

Nonhomologous end joining (NHEJ)

It is responsible for repairing double-stranded DNA when it breaks. This repair mechanism occurs in mammalian cells when the DNA damage is out of replication fork and not in S-phase. It rejoins the broken ends, free of DNA, and often involves loss of a DNA sequence in free DNA ends. To ensure accurate repair, this system is based on short homologous sequences called micro homologies, present in single-stranded tails at the ends of DNA that must be joined. If these sequences are compatible, repair is usually correct. *XRCC4 XRCC5* mutations and this system in *XRCC4* are associated with embryonic lethality in mice specimens. This can be mitigated by crossing the *XRCC4* knockouts with p53 mutants, suggesting that lethality is a result of p53 mediated apoptosis. Some polymorphisms of *XRCC4* have been described related to high risk of lung cancer, colon cancer and thyroid [24-26].

Homologous recombinational repair (HRR)

It repairs double-stranded DNA replication fork and when cells are in S phase. It requires the presence of an identical or nearly identical sequence to be used as a template to repair the tear. This system allows that a damage chromosome is repaired using a sister chromatid (available in G2 phase after DNA replication) or homologous chromosome as a template. This system can be further subdivided into the gene conversion and single-strand annealing subpathways.

Examples of proteins belonging to this system are *BRCA1*, *BRCA2* and *RAD 51*.

The *BRCA1* gene is located on chromosome 17 (17q21). More than two hundred mutations have been identified in the *BRCA1* gene. Depending on the mutation, an abnormally short version of the *BRCA1* protein is found, or any protein is made from one copy of the gene. Other *BRCA1* mutations change single protein building blocks (amino acids) in the protein or delete large segments of DNA from the *BRCA1* gene. It is thought that a defective or missing *BRCA1* protein is unable to repair damaged DNA or fix mutations that occur in other genes, which is the main cause of its association with increased cancer risk (particularly breast cancer and ovarian cancer).

BRCA1 and *BRCA2*-deficient cells are highly sensitive to ionizing radiation and display chromosomal instability, which is likely to be a direct consequence of unrepaired DNA damage.

BRCA1 has a role in signalling DNA damage and cell-cycle checkpoint regulation; however *BRCA2* has a more direct role in DNA repair itself [3]. It is thought that *BRCA2* promotes genomic stability through a role in the error-free repair of double-strand breaks.

The interaction of *BRCA1*, *BRCA2* and RAD51 suggest a functional link between the three proteins. The physical interaction between *BRCA2* and *RAD51* is essential for error-free double-strand breaks. *BRCA2* is required for the localization of *RAD51* to sites of DNA damage, where *RAD51* forms the nucleoprotein filament required for recombination [3].

There are more than 600 mutations in the *BRCA1* gene, many of which are associated with an increased risk of cancer. Hypermethylation of its promoter has been associated with the development of breast cancer, which is believed to be understood as a mechanism to inactivate the expression of *BRCA1*.

In *BRCA1* but not *BRCA2* associated tumors, additional molecular genetic abnormalities that are commonly found include mutations in p53 and PTEN tumor suppressor genes.

Data also suggest a potential role of *BRCA1* and *BRCA2* mutations in sporadic breast and ovarian cancers, in particular triple-negative, or medullary histology. The genomic instability of *BRCA1* and 2 deficient cells in hereditary and triple-negative breast cancer provide an opportunity for therapeutic development, especially drugs that target DNA repair pathways. This includes platinum-type drugs that generate double-stranded DNA breaks or PARP inhibitors, which are involved in the repair of DNA single-strand breaks.

The population frequency of mutations in the *BRCA1* and *2* genes is generally estimated to be 1/800 to 1/1000 per gene [27]. Higher prevalence is found among individuals with a personal history of breast cancer and/or a family history of breast and ovarian cancer, especially if associated with young age on onset, multiple tumors, and involvement of male family members affected with breast cancer.

Cancer syndrome hereditary breast and ovarian cancer due to *BRCA1* and *2* mutations in the germ line, with an autosomal dominant inheritance pattern is characterized by a high risk of breast cancer, between 50 and 85%, and 15-40% of ovarian cancer. Individuals suspected of having a hereditary predisposition to breast and/or ovarian cancer based upon personal and family history should be referred to a genetic counsellor for formal risk assessment.

These last two mechanisms, homologous recombination and nonhomologous are extremely crucial for maintaining cell survival, proliferation and genomic stability. The breaking of the double strands of DNA (DSB), substrate of both mechanisms is the most lethal DNA lesions, and appears with a high frequency. Although it appears that cells can tolerate a small amount of irreparable DNA damage, a single break in the double-stranded DNA can be cytotoxic or in some cases sufficient to trigger apoptosis; also it has been established as a causal link between DSB and genomic instability. As noted previously, it has been shown that defects in both pathways lead to cancer.

Cell cycle checkpoint control

This system delays the cell cycle progression in response to DNA damage for the cell to have a half "extra" and try to repair the damage before entering a critical phase that can become lethal. *P53* belongs to this system.

The *p53* gene is located on the short arm of chromosome 17 (17p13), is essential to induce cell response to DNA damage, arresting the cell cycle in case of mutation [28].

The transcription factor *p53* has several important functions:

a) Cell cycle arrest in G1 / S transition: it is due to *p53*-dependent transcription of the CDK inhibitor called CDKN1A/p21; p21 inhibits CDK-cyclin complexes and prevents the phosphorylation of pRb, so that the factor inactivates E2F transcription and prevents the progression of the cell into S phase. Thus, the cell has time to repair the damage to DNA.

b) Activation of DNA repair enzymes: a transcriptional target genes, *p53R2* encodes a ribonucleotide reductase, important for DNA replication and repair. Likewise, it also interacts directly

with AP endonuclease and DNA polymerase involved in excision repair. *P53* protein also induces GADD45 (growth arrest and DNA damage), which helps repair DNA. If the damage is fully repaired, *p53* stimulates the synthesis of Mdm2, activating its self-destruction and cell cycle progression, but if the cell is not able to repair the damage, apoptosis or senescence enter both processes induced by *p53*.

c) Entry into apoptosis: *P53* activates the expression of genes such as BAX pro-apoptotic or PUMA. The process of how the cell "chooses" to repair their DNA or enter apoptosis is not entirely clear, it appears that *p53* has higher affinity for the promoters of DNA repair genes than pro-apoptotic genes. Thus, *p53* levels would be crucial to this. At first trigger DNA repair, but if this is ineffective and *p53* levels continue to rise, it would activate pro-apoptotic genes.

d) Entry into senescence: This is a permanent stop in the cell cycle usually irreversible. The changes are not yet fully known, but appear to involve epigenetic chromatin modifications as heterocromatina training block in the proliferation activating genes regulated by E2F. The entry into senescence can be induced by the presence of different types of stress such as hypoxia, telomere shortening or oncogenic signaling.

It is vital to the strong regulation of the cellular concentration of *p53*, because even if *p53* is a tumor suppressor, high levels can accelerate the aging process by excessive apoptosis. Its main regulator is Mdm2, which can activate *p53* degradation by ubiquitination.

p53 is able to detect the presence of cell damage, but two key sensors of DNA damage are two related kinases: ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and RAD3 related); they were initially identified in patients with ataxia-telangiectasia, which have a high incidence of cancer as they are not able to repair certain DNA lesions. They detect different types of lesions in the DNA, but they activate similar signaling pathways, phosphorylates several proteins involved in DNA repair and *p53*. When *p53* is phosphorylated, it is released from its association with Mdm2, acting as a transcription factor and increasing the expression of proteins such as GADD45 (DNA repair), p21 (inhibition of progression through the cell cycle) and BAX (promotion of apoptosis). Likewise, *p53* activates transcription of the family of mir34 microRNAs, small RNA molecules that prevent the translation of specific mRNAs, inducing major stop on the cell cycle and apoptosis.

Mutations in *p53* are presents in more than 50% of the global of the tumors.

P53 mutations have been associated with lung cancer more often than any other genetic abnormality. Rates of detected mutations range from 20 to 60 percent of all cases of NSCLC, and they are more common in cancers associated with smoking, such as SCC, compared to adenocarcinomas. Loss of heterozygosity (deletion of genetic material from one 17p allele) also occurs in the bronchial tissue of approximately 20 percent of smokers, possibly identifying a population at high risk for developing malignancy. Particularly, specific hotspot mutations in *p53* that result in substitution of thymidine for guanine residues are commonly seen in smoking-induced cancers. The relationship between *p53* mutations and survival in patients with NSCLC remains unclear despite years of study. At least two meta-analyses have explored the influence of *p53* mutations or over expression on prognosis. In the first one, the authors suggests that an abnormal *p53* status had an unfavourable impact on survival in any stage NSCLC, but in the second, the negative prognostic impact of *p53* alteration was highly significant in patients with adenocarcinoma but not in those with SCC.

Most of these studies are retrospective, limited by small size, with heterogeneous populations and with insensitive *p53* mutation detection techniques. It is also important that reported concordance levels between immunohistochemistry and mutational analysis for *p53* mutations are only approximately 65%. Certain p53 mutations may result in either no *p53* expression or low levels of expression that are not detectable by IHC. Bodner et al. published an article in which less than one-half of gene mutations were associated with a positive IHC *p53* protein.

It is also described as a relationship between aberrant *p53* expression and angiogenesis in patients with NSCLC.

Different tumors with a high percentage of *p53* mutations are colorectal cancer, ovarian cancer, secondary glioblastoma, non-melanoma skin and head and neck.

Apoptosis

It is programmed cell death or "cell suicide". It starts as a result of severe damage in DNA that prevents the cell to perform its normal functions. It may begin in the final third of G1 (to prevent a damaged cell to S phase pass, so that mutations may not play during DNA replication) and G2 phase (to prevent cells that have not reached to maturity enter mitosis). Apoptosis occurs by chromatin condensation, cytoplasmic shrinkage, plasma membrane blebbing and the division of DNA. In this mechanism, several agents act to highlight the complex cysteinyl-aspartate proteases (caspases) and *Bcl-2* family.

Apoptosis in mammalian cells is mediated by a family of cysteine proteases, caspases. These proteins are present in cells in an inactive form, when the initiator caspases (8 and 9) are activated by oligomerization, the proto-digested effector caspases (3, 6 and 7), activating them. The active effector caspases digest a specific group of cellular substrates, which regulate the orderly destruction of the cell. Subsequently, cell membranes are broken, cytoplasm and cytoskeleton collapse, the cytosol is consumed, the chromosomes are degraded and the nucleus is fragmented.

Apoptosis can be triggered by various mechanisms, both internal (DNA damage, mediated by p53 signaling imbalance, insufficient growth factors or hypoxia, HIF-mediated) and external (physical or chemical agents). And so, one of the two pathways that control apoptosis is activated:

- The extrinsic pathway is activated by the binding of different signals to the membrane receptors of the superfamily of receptors for tumor necrosis factor, and Fas. This pathway is used by cytotoxic T lymphocytes, which present on their surface Fas ligand to induce apoptosis in certain cells.

- The intrinsic pathway or mitochondrial involves permeabilization of the outer mitochondrial membrane, allowing cytoplasmic release of cytochrome c. This pathway is controlled by proteins of the Bcl-2 family.

Bcl-2 is a family of proteins anti-apoptotic made up of about 25 proteins that regulate mitochondrial permeabilization processes [29]; they are a key point in the intrinsic pathway of apoptosis. Its name

comes from the proto-oncogene Bcl-2 (B-cell lymphoma 2), a member of a group of proteins described in studies of the translocation between chromosomes 14 and 18 observed in follicular lymphomas. Further investigation described the existence of two homologous pro-apoptotic subfamilies, Bax and BH3.

In viable cells, BAX and BAK (both family proteins BAX, proapoptotic) are in monomeric form [30], the first anchored to the cytosolic face of various organelles and the second within the mitochondria. Before a cell death signal, BAX is inserted into the outer membrane of mitochondria as homooligomer and BAK undergoes a conformational change that includes its oligomerization and permeabilization of the mitochondrial outer membrane with the release of intermembrane space factors such as cytochrome c. Cytochrome c, once in the cytosol, it activates a protein complex called "apoptosome", which directly activates caspase-9. Once caspase-9 is activated, it activates the effector caspases such as caspase-3, triggering the final stages of apoptosis. This process occurs equally in mediating the endoplasmic reticulum calcium release.

The BH3 subfamily members function as initiators of the pathway that selectively integrate the various specific responses death and survival.

Bcl-2 is highly expressed in neoplastic cells in over 90% of follicular lymphoma patients [31]. This high expression of *Bcl2* is due to a specific chromosomal translocation t (14;18) (q32;21) that generates a fusion between the immunoglobulin heavy chain enhancer on chromosome 14 and the *Bcl-2* gene on chromosome 18; this was one of the earliest chromosomal translocations related to cancer development to be discovered. Normally, most B-cells should be terminated via apoptosis if they are not challenged by specific antigens. With the overexpression of *Bcl-2*, follicular lymphoma cells are able to overcome normal apoptotic signals and avoid termination. Therefore, the prolonged life span of follicular cells due to this defect in apoptotic elimination contributes to the development of follicular lymphoma.

Bcl-2 family, other than in the follicular B-cell lymphoma, is involved in a number of cancers, including melanoma and breast carcinoma, prostate and lung. Its expression was associated with good prognosis in non-small-cell lung cancer. Cytotoxicity of many chemotherapeutic agents is induced through the Bcl-2 apoptotic pathway; overexpression of *BCL2* is associated with increased resistance to these agents.

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