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Principle of Oncology

Introduction to oncogenetics :

Oncogenetics is primarily a predictive and preventive science in the field of genetics that focuses mainly on hereditary cancers. It's a fascinating branch of genetics that delves into the relationship between genes and cancer. It explores how genetic variations can influence the risk of developing cancer, as well as how this information can be utilized for cancer prevention, diagnosis, and personalized treatment.
At the core of oncogenetics is the identification of specific genes, known as oncogenes, which play crucial roles in the cellular transformation process leading to cancer. These oncogenes can be activated by various genetic mutations, whether inherited or acquired throughout life. Similarly, certain genetic variations, known as cancer susceptibility variants, may increase predisposition to specific types of cancer.

Understanding oncogenetics also involves studying the complex interplay between genes and the environment, as external factors such as exposure to carcinogens or lifestyle choices can interact with genetic predisposition to influence cancer risk.

The advent of high-throughput sequencing technologies has revolutionized the field of oncogenetics, enabling in-depth analysis of the genomes of cancer patients. This has opened new avenues for personalized medicine, where genetic information can guide the selection of the most effective and individualized treatments, as well as the implementation of early screening programs for individuals at risk.

In summary, oncogenetics is an essential discipline in the fight against cancer, offering promising insights for better understanding the disease and the development of more targeted and effective prevention and treatment strategies.

A significant proportion of cancers are linked to the presence of a hereditary genetic alteration, meaning that a gene anomaly has been present since birth and can therefore be passed on to offspring.

This module aims to shed light on the study of the genome of human tumor cells, which has revealed a much larger number of genetic and epigenetic alterations than previously anticipated. These changes occur in various molecular mechanisms, thus controlling the proliferation and survival of abnormal cells during tumor progression.

1. Cancer disease, oncogenes and tumor suppressor genes:

Cancer is characterized by the anarchic multiplication of cells, due to mutations that accumulate over time. These mutations affect various genes, including oncogenes and tumor suppressor genes. However, our genome is equipped with several repair systems to remedy the various lesions that can lead to the development of a cancerous cell.

This repair system calls on numerous genes which are activated not by mutagenesis but by much lower doses of carcinogenic agents, which leads us to say that carcinogenesis is not only initiated by genome lesions affecting a few specific targets (proto-oncogenes, suppressor genes, etc.) but also by the relationship between the initiated cells and their microenvironment. In this way, the carcinogenic process is counterbalanced by effective defense mechanisms in the cell, tissue and organism (Franco et al. 2005).

However, mutation accumulation becomes such that the DNA repair system (which plays a crucial role in preserving genome integrity) is no longer able to correct DNA damage. When these repair mechanisms fail, they can also contribute to the development of cancer.

a. Oncogenes :

Oncogenes are the altered version of proto-oncogenes. The expression of proto-oncogenes is essential during regulated growth, such as embryogenesis, wound healing, regeneration of damaged liver and stimulation of cell mitosis by growth factors. Proto-oncogenes are found in diverse species such as yeast, Drosophila and man.

These genes encode growth factors, growth factor receptor tyrosine kinases, signal transduction regulatory proteins, non-receptor tyrosine kinases, serine/threonine kinases and transcription factors. Encoded proteins play a crucial role in cell growth and differentiation (Hunter 1991) and in apoptosis or programmed cell death (Hockenbery et al. 1990).

- **Activation of proto-oncogenes :**

The mechanisms of proto-oncogene activation are not exclusive to a particular proto-oncogene, and they vary considerably according to the genetic aberrations they cause. What they do have in common, however, is aberrant gene expression, regulation or product.

The inability of a cell to modulate the expression or function of a proto-oncogene results in a cell that does not respond to the intracellular and extracellular signals that normally regulate cell proliferation and differentiation.

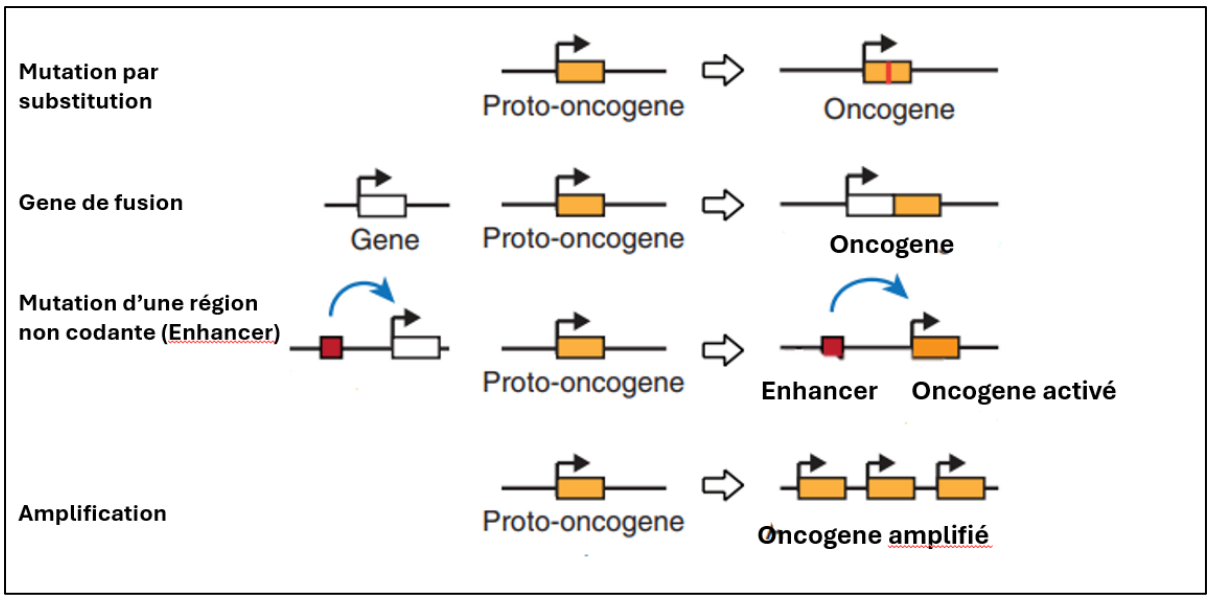


Figure 1 Examples of some proto-oncogene activation mechanisms (Hnisz et al. 2016)

b. <u>Tumor suppressor genes</u>

The function of tumor suppressor genes is to inhibit growth during uncontrolled cell division, and to stimulate cell death in order to maintain equilibrium. Some of these genes are also involved in DNA repair processes, helping to prevent the accumulation of mutations in cancer-related genes. In this way, tumor suppressor genes act as "brakes" to stop cells before they become cancerous.

The study of mutations in tumor suppressor genes, which cause the loss of their functions, provides essential answers in the development of cancer cells.

Inactivation of tumor suppressor gene :

- Heterozygosity loss

Loss of heterozygosity is of great importance in the field of genetics. It can affect the frequency and prevalence of genetic diseases within a population. By reducing genetic diversity, loss of heterozygosity can increase the risk of transmission of recessive hereditary diseases. It can also play a role in the development of cancer, as certain tumor suppressor genes can be affected by this phenomenon. Understanding the mechanisms and consequences of heterozygosity loss is therefore essential to progress in the field of genetics. During this process, a heterozygous cell receives a second response in its remaining functional copy of the tumor suppressor gene, thus becoming homozygous for the mutated gene. Mutations that inactivate tumor suppressor genes are often point mutations or small deletions that disrupt the function of the protein encoded by the gene; chromosomal deletions or breaks that delete the tumor suppressor gene; or cases of somatic recombination in which the normal copy of the gene is replaced by a mutant copy. Understanding the mechanisms and consequences of loss of heterozygosity is therefore essential to progress in the field of genetics.

2. Cancer diagnosis: pathologicalanatomy techniques

The diagnosis of cancer often involves a series of tests and procedures to identify the presence of cancer cells from a tissue or cell sample.

In pathological anatomy, the following techniques are commonly used:

- **Cytopathological examination:** Analysis of individual cells taken from tissue or body fluid samples.
- **Histopathological examination:** Detailed study of tissue, in which cells are examined in their tissue context.
- **Biopsy:** Removal of a tissue sample for microscopic examination, often considered the most reliable means of diagnosing cancer.
- **Flow cytometry:** Technique for analyzing the physical and chemical properties of cells.

- **Molecular techniques:** Include PCR (polymerase chain reaction), FISH (fluorescent in situ hybridization) and NGS (next-generation sequencing), which can detect genetic alterations specific to cancers.
- **Liquid biopsy:** A blood test that can detect tumor DNA fragments circulating in the blood

These techniques are complementary and can be used together to obtain an accurate diagnosis. The pathology report provides the definitive diagnosis and is also used for **staging**, describing the extent of cancer in the body, and to help plan treatment.

3.Classification of solid tumors and differentiation :

a. TNM classification

Practitioners use the TNM classification to classify malignant tumors. TNM is mainly used for solid tumors, and can help in the prognostic staging of cancer. The system is based on evaluation of the tumor, regional lymph nodes and distant metastases, as detailed below.

T- Tumor. Used to describe the size of the primary tumor and its invasion into adjacent tissues. T0 indicates no evidence of tumor, while T1-T4 are used to identify tumor size and extension, with progressive enlargement and invasiveness from T1 to T4. T values are assessed differently depending on the anatomical structures involved. For example, T1 indicates invasion into the submucosa in colorectal cancer, while T4 indicates tumor extension through all layers of the colon and invasion of the visceral peritoneum or adjacent structures. T2 indicates invasion of the muscularispropria, and T3 is invasion of the subserosa. Tis identifies carcinoma in situ. Tx is used when the tumor cannot be evaluated.

N- Nodes. Used to describe the regional lymph node involvement of the tumor. Lymph nodes function as biological filters, as fluid from body tissues is absorbed into lymphatic capillaries and flows to lymph nodes (Sapin 2007).

N0 indicates no regional lymph node spread, while N1-N3 indicates some degree of lymph node spread, with spread progressively distal from N1 to N3.

N values are assessed differently for specific tumors and their regional lymph node drainage. In colorectal cancer, N1 indicates the involvement of 1 to 3 regional lymph nodes. N2 may be

4 to 6 regional nodes, while N3 indicates 7+ regional nodes involved. Nx is used when lymph nodes cannot be evaluated.

M- Metastasis. Used to identify the presence of metastases at a distance from the primary tumor. Metastasis occurs when the tumor spreads beyond the regional lymph nodes. A tumor is classified as M0 if no distant metastases are present, and M1 if there is evidence of distant metastases. This classification can be further subdivided by tumor to provide more detailed information. In the colorectal cancer classification, M1a indicates spread to 1 zone, M1b is spread to 2+ zones and M1c means spread to the peritoneal surface. Peritoneal carcinosis, in particular, is a poor prognostic factor for colorectal carcinoma (Skotnicki et al. 1976) (Lotfollahzadeh et al. 2024).

Difference between grade and stage of cancer

Cancer grade is a description of the microscopic appearance of tumor cells and tissues. Low-grade tumors have relatively normal-looking cells and tissue structures. These tumors are considered well-differentiated. Higher-grade tumors have more abnormal-looking cells and abnormally structured tissue. High-grade tumors are generally more aggressive and have a poorer prognosis. They are described as poorly differentiated. High-grade tumors are said to be undifferentiated.

Cancer staging is a description of the macroscopic appearance of the tumor. It can be described in terms of tumor size, invasion, spread to local lymph nodes or distant metastases. Somestagingsystemsalsoincludetumor grade.

b. <u>Differentiation antigen</u>

Any molecule that can be recognized by the immune system is considered an antigen. Many tumor cells produce antigens, which may be released into the bloodstream or remain on the cell surface. These have been identified in most human cancers, including Burkitt's lymphoma, neuroblastoma, melanoma, osteosarcoma, renal cell cancer, breast cancer, prostate cancer, lung cancer and colon cancer. Tumor-specific antigens (**TSA***are present only on tumor cells*) and tumor antigens (*relatively specific to tumor cells*.) are generally portions

of intracellular molecules expressed on the cell surface as part of the major histocompatibility complex. However, many antigens that are selectively expressed on the surface of tumor cells are not associated with the major histocompatibility complex and may be candidates for therapeutic targeting.

These antigens can be a reliable diagnostic tool, as tumor immunodiagnosis involves the use of specific tumor-associated antigens. Indeed, tumor-associated antigens can facilitate the diagnosis of many tumors and sometimes determine response to treatment or highlight recurrence. An ideal tumor marker should :

- Be released only by tumor tissue
- Be specific to a given tumor type
- Be detectable at low levels of tumor cell burden
- Have a direct relationship with tumor cell burden
- Be present in all patients with a tumor.

Although the majority of tumors secrete antigenic macromolecules detectable in the circulation, no tumor marker has all the characteristics required to be used in an early diagnosis or screening program with sufficient specificity or sensitivity. For example, **Prostate-Specific Antigen (PSA)**, a glycoprotein localized in the epithelial cells of the ducts of the prostate gland, can be detected at low concentrations in the serum of healthy men. Based on an adequate upper limit of normal values, tests using monoclonal antibodies find elevated plasma PSA concentrations in almost 90% of patients with advanced prostate cancer, even in the absence of definite metastatic tumor. It is more sensitive than prostatic acid phosphatase. However, because PSA is elevated in other conditions (e.g. benign prostatic hyperplasia, prostatitis, recent instrumentation of the genitourinary tract), it is less specific. PSA can be used to monitor recurrence after prostate cancer diagnosis and treatment (Kelley et al. 1998).

Chapter II
General oncogenetics

1. Immune response genes and cancer :

The development of a tumor within an organism is closely linked to its immune system. An immunosurveillance process protects the host from the development of a tumor focus. However, it is also recognized that the immune system facilitates tumor progression, notably by shaping the immunogenic phenotype of the tumor during its development. The immune system therefore plays a dual role in the complex relationship between host and tumor.

The immune system thus appears to be involved in both host protection and the establishment of the cancerous focus, both preventing and contributing to tumor development. Dunn GP et al. have summarized the various studies carried out on this subject by constructing a model illustrated **in figure 2**. The immune establishment of cancer can be summarized in three stages: elimination, equilibrium and escape.

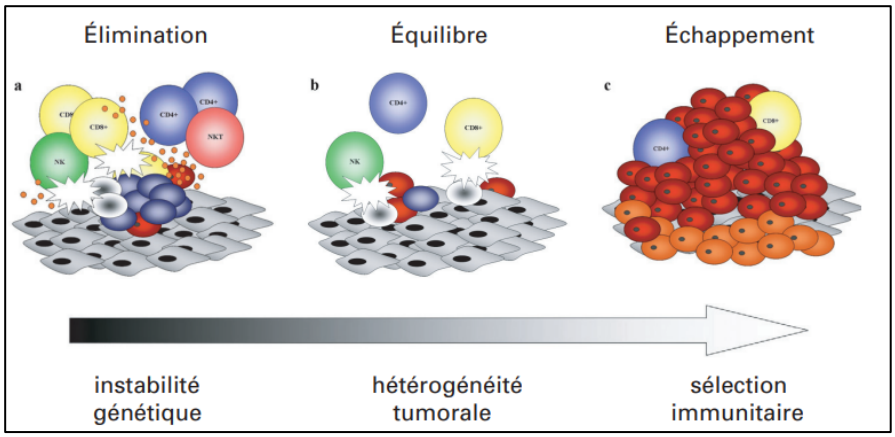


Figure 2(**a**) Immunosurveillance leads to elimination. (**b**) Equilibrium phase in which the immune system helps generate a resistant tumor cell clone. (**c**) Uncontrolled proliferation of the tumor clone, evading the immune response. Tumor cells (**blue**); tumor cell variants (**red and orange**); stroma (**gray**). Lymphocyte populations are represented by distinct colors. Small orange circles represent cytokines and white flashes, the cytotoxic activity of lymphocytes on tumor cells.

The concept of immunosurveillance comes into play during the elimination phase, when the immune system enables the tumor to be destroyed. During this phase, various immune system players are recruited to the tumor site to limit its development. Once the majority of tumor cells have been eliminated by the immune system, a phase of dynamic equilibrium is established between the immune system and the surviving tumor variants.

This phase can be seen as a period of Darwinian selection, during which numerous and rapid genetic mutations take place, leading to the development of a clone resistant to attack by the immune system. This period, considered the longest of the three, can extend over several years. In the final phase, the tumor variant(s), having acquired insensitivity to detection and/ or elimination by the immune system, begin their phase of uncontrolled growth. This leads to the development of cancer.

Here are a few examples of genes that play a key role in this response:

- ***Genes that code for tumor antigens :***

Genes involved in the production of these antigens, such as tumor-specific antigens (TSA) and tumor-associated antigens (TAA), are important in the recognition of tumor cells by the immune system. In a study by Coulie et al, a gene coding for one of these antigens, designated LB33-B, was discovered and named MUM-1. This gene has no significant homology with known genes. It is expressed in most normal tissues. The MUM-1 gene present in melanoma cells differs from the MUM-1 gene found in normal cells (of the patient selected in this study) by a point mutation. This mutation modifies an amino acid in the antigenic peptide presented to T lymphocytes by the HLA-B44 molecule. In addition, it was observed that the messenger RNA encoding the LB33-B antigen was incompletely spliced, retaining one of the introns of the MUM-1 gene. (Coulie 1995).

- ***Immune checkpoint genes :***

Genes such as PD-1, PD-L1, CTLA-4, and others, regulate the activation and deactivation of T cells, playing a key role in regulating the immune response against cancer. Programmed death ligand 1 (PD-L1) is the major ligand in programmed death 1 (PD-1), a co-inhibitory receptor that can be constitutively expressed or induced in normal myeloid, lymphoid, epithelial cells and in cancer. Under physiological conditions, PD-1/PD-L1 interaction is essential for the development of immune tolerance, preventing excessive immune cell activity that can lead to tissue destruction and autoimmunity. PD-L1 expression is an immune evasion mechanism exploited by various malignant tumors and is generally associated with a poor prognosis. PD-L1 expression is also suggested as a predictive biomarker of response to anti-PD-1/PD-L1 therapies; however, conflicting evidence exists as to its role depending on histotype. Over the years, anti-PD-1/PD-L1 agents have gained momentum as novel

anticancer therapies, inducing durable tumor regression in many malignancies, including metastatic lung cancer, melanoma and many others. In this review, we discuss the immunobiology of PD-L1, with particular emphasis on its clinical significance in malignancies (Kythreotou et al. 2018).

2. Viruses and Merckel's tumor cancer :

Oncogenic viruses generally maintain chronic infections in which there is little or no production of viral particles, and which last for the lifetime of the infected individual. These mechanisms of viral persistence and/or latency are biologically compatible with the carcinogenic process, as they avoid the cell death most common in acute lytic infections, while keeping the infectious agent hidden from the immune system. Viral persistence in the host is achieved by integrating the viral genome into the cellular genome or by expressing viral proteins that also separate the viral genome into daughter cells during cell partition. Both mechanisms ensure that the virus is not lost during cellular replication. Viral persistence is generally characterised by the expression of proteins that control cell death and proliferation; in this way, oncogenic viruses nourish the infection of a controlled number of cells, establishing a balance between virus and host, thus preserving the integrity of both. Cellular transformation is probably not an evolutionary viral strategy, but rather a biological accident that rarely occurs in the virus-host interaction. Cancer leads to the death of the host and therefore also represents the end of the virus. The existence of viral oncogenes is explained by the fact that they are part of the viral persistence mechanisms, which can only lead to cancer under altered conditions. All virus-associated tumours result from the cooperation of various events, involving more than just the persistent mechanisms of infection and viral transformation. Additional oncogenic attacks are necessary for complete transformation. The appearance of mutations altering the expression and function of viral and/or cellular oncogenes is necessary in the carcinogenic process, and so an increased mutation rate in infected cells compared with normal cells is frequently observed (Loeb, Springgate, et Battula 1974).Cells latently infected with oncogenic viruses could be more susceptible targets for further oncogenic attacks; for example, as a result of smoking, a diet low in fruit and vegetables and/or increased exposure to environmental oncogens. All these insults, together with the host genetic component behind the inflammatory responses

triggered by the infection itself, lead to cellular transformation and the development of cancer.

- Polymavirus and Merckel's tumour

The polyomavirus genome is a small circular double-stranded DNA consisting of just over 5,000 base pairs (**Figure 3**). This circular genome is associated with cellular histones, forming a nucleo-protein structure reminiscent of a plasmid or mini-chromosome. From a phylogenetic point of view, there are three polyomavirus genogroups: primate polyomaviruses (SV40, Baboon PyV, BKPyV and JCPyV), murine polyomaviruses (MuPyV, Hamster PyV) and avian polyomaviruses (Avian PyV, Goose PyV). The viral genome can be divided into three regions: two conserved coding regions, an early and a late region, separated by a third non-coding variable region that regulates the origin of replication.The region that is transcribed and translated in the early stages of the replication cycle represents about half of the genome (around 2.5 kb) and codes for regulatory proteins also known as T antigens (tumor antigens). These proteins are the result of alternative splicing from a common messenger, leading to the separate production of the small T antigen (t-Ag) and the large T antigen (T-Ag). For non-primate polyomavirusgenogroups, a third intermediate T antigen may be produced, the "middle T antigen". The late region codes for capsid structural proteins. All three proteins are derived from the same messenger, and VP2 and VP3 are translated from the same messenger using a different initiation codon. This region also codes in the primate group for a small protein called agnoprotein, which is thought to be involved in encapsidation, but which may also play a role alongside the T antigen in regulating the viral and cell cycle. The non-coding area separating the early and late regions contains an origin of replication, a TATA box motif, binding sites for T antigen and cellular transcription factors, promoters and strong enhancers. This region, which is highly variable from one virus to another, could confer selective advantages in terms of transcription and replication of the virus for its host.

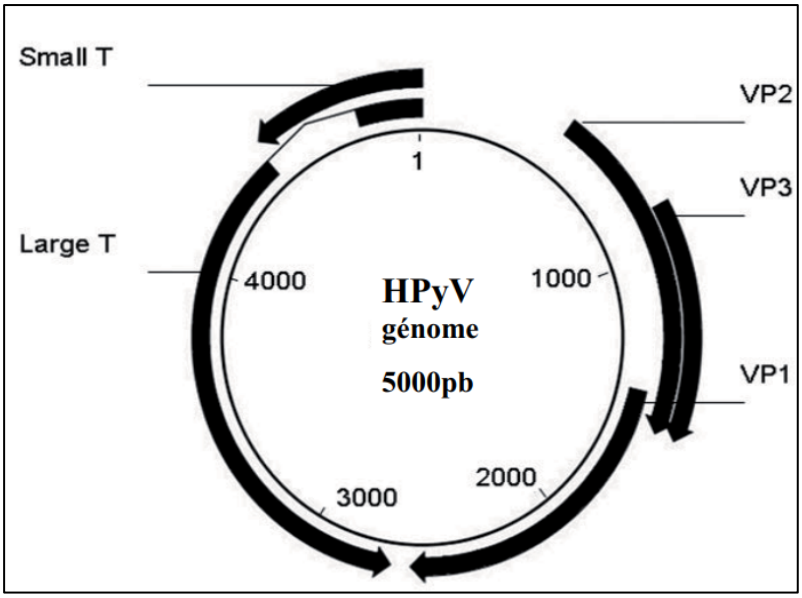


Figure 3 Schematic representation of the circular genome of human polyomavirusesoma virus humains (Foulongne 2012).

Historically, only the JCV and BKV polyomaviruses were thought to infect humans, but next-generation sequencing techniques have enabled the identification of at least nine other members in humans, including MCPV. MCPV was identified in 2008 in an aggressive skin cancer called Merkel cell carcinoma (MCC) (Feng et al. 2008). Virtually the entire adult population of the world is infected with MCPV. Evidence supporting the involvement of this agent in the carcinogenesis of MCC includes the presence of MCPV genomes in approximately 80% of tumours but not in healthy tissues, and clonal integration of the viral genome (Carter et al. 2009). Oncogenic transformation of MCPV may result from a loss of immune surveillance, as MCC occurs mainly in immunosuppressed individuals.

The MCPV genome is inserted into the host genome during viral carcinogenesis. Integration is characterised by the preservation of virus-induced cell proliferation functions while suppressing viral replication; the latter is probably due to the deletion of certain regions of the viral T antigen gene (Hodgson 2005). Viral integration also promotes host resistance to cell death, favouring viral persistence in the latent state (Moens, Van Ghelue, and Johannessen 2007). This is a significant difference between the presence of the virus in MCC and in non-tumour tissues. Some of these viral proteins show homology in functional domains with tumorigenic polyomaviruses from non-human species. For example, like SV40, MCPV T antigens are generated by differential splicing to produce large T antigens and small T

antigens (Shuda et al. 2009). The large T antigen has the structural motif that inactivates pRb (Shuda et al. 2008), and the T antigen is generally expressed in the MCC, and even in its truncated form maintains the pRb inactivation domain intact (Houben et al. 2012). Inactivation of the T antigen in MCC cell lines leads to cell death, reinforcing the causal role of MCPV in MCC (Houben et al. 2010). In addition, the small T antigen retains the AKT/ mTOR activation domain, which is responsible for the loss of contact inhibition and promotes substrate- and serum-independent growth (Shuda et al. 2011).

3. Monofactorial and multifactorial genetic predisposition:

A genetic disease is monofactorial when its emergence depends on the mutation of a single gene, even though modulating genes can accentuate or attenuate the clinical phenotype, as can variations in the environment. This is why single-factor diseases are also referred to as monogenic or Mendelian, because their mode of transmission obeys Mendel's laws defined for variation in the single gene involved in their emergence.

In contrast, a disease is multifactorial when its emergence depends simultaneously on several causes, both genetic and environmental, making its heredity 'complex' and unpredictable, totally incompatible with the transmission models defined for a monofactorialdisease.The involvement of genetic factors in the emergence of a multifactorial disease is attested by two types of observation.

The clustering of a large number of cases within families that share common genes, but also environmental factors; the greater concordance between monozygotic twins (MZ) than between dizygotic twins (DZ) or germans (60% versus 25% for Alzheimer's disease, or 18% versus 8% for Crohn's disease), illustrating this 'genetic component' of the disease. The involvement of environmental factors in the emergence of a multifactorial disease is also evident from the fact that families share a common environment and, above all, from the imperfect concordance between MZ twins despite their genetic identity (60% for Alzheimer's, 18% for Crohn's), which should be 100% if environmental factors were not involved.

- Single-factor (monofactorial) diseases :

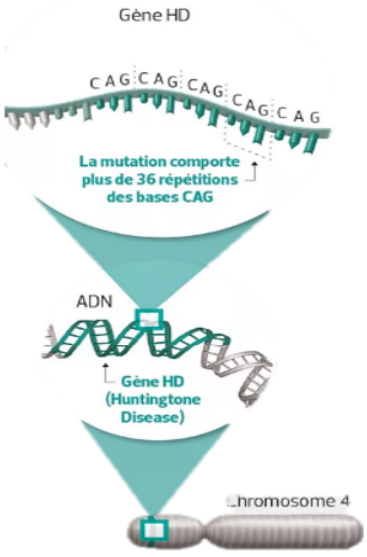
Result from the mutation of a single gene, the transmission of which follows Mendel's laws. Of the 23 pairs of chromosomes in the nucleus of a cell, 22 pairs, called autosomes, are identical in morphology in both sexes, and the last pair, represented by the sex chromosomes,

consists of two X chromosomes in women and one X and one Y chromosome in men (Braekeleer 2000).

Since chromosomes come in pairs, the gene exists in two copies, each of which, called an 'allele', may be different from the other (heterozygous) or identical to it (homozygous). So, depending on whether the gene mutation is located on an autosome or on a sex chromosome, and whether its effect occurs in the presence of a single allele (dominant effect) or of both alleles of the gene (recessive effect), it is possible to distinguish four modes of appearance of a single-factor disease:

- Autosomal dominant diseases are expressed as soon as one of the two alleles carried on a non-sex chromosome is mutated (e.g. Huntington's disease).

The disease is caused by the abnormal repetition of the CAG base sequence on the HD gene located on chromosome 4; this abnormal sequence can comprise from 55 to 100 repetitions. This gene produces the Huntingtin protein, which has a protective role for the brain.



- Autosomal recessive diseases occur if, and only if, the two homologous genes both carry the mutation. Heterozygous individuals are healthy carriers of a mutated gene. Such a defective gene will never make them ill because the other chromosome carries the normal gene. However, heterozygotes run the risk of passing the disease on to their children if they marry another heterozygote who is a healthy carrier of the same mutated gene. The risk of a heterozygous couple having a child with the disease is then 25%16 (e.g. cystic fibrosis fig 4).

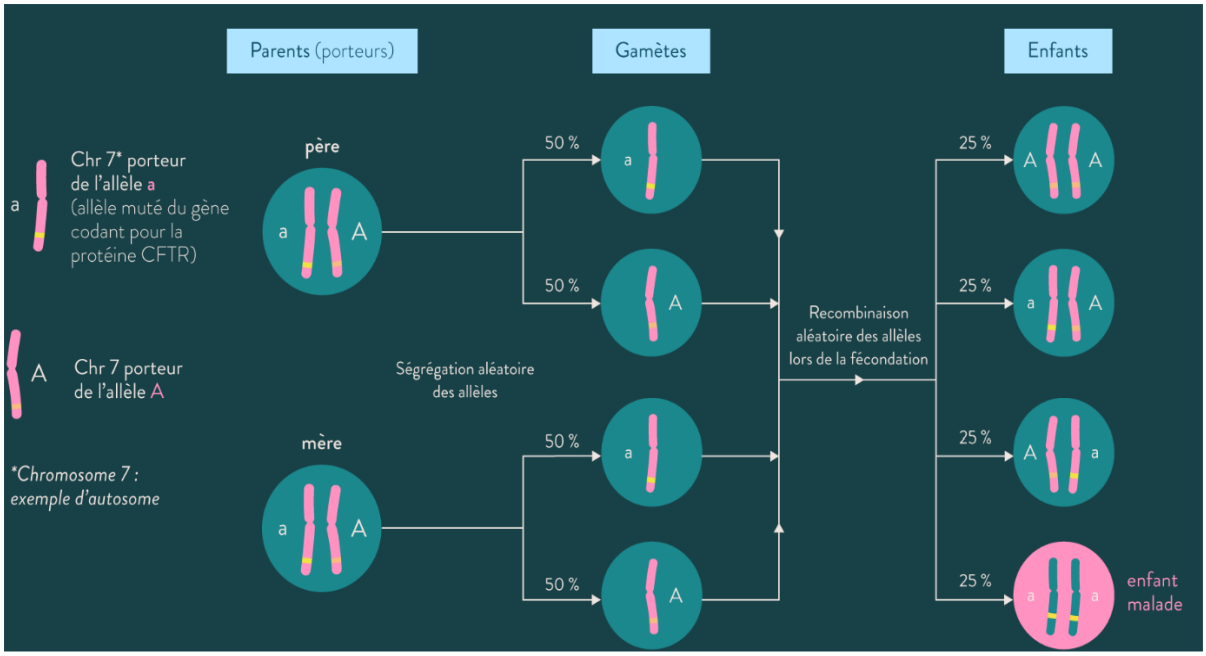


Figure 4 transmission scheme for cystic fibrosis

- X-linked diseases are generally recessive:

As women have two X chromosomes, they will not be affected by the disease if only one allele of the two X chromosomes has mutated. In this case, they are nevertheless carriers of the disease which affects their son who has inherited the defective gene carried on his single X chromosome (e.g. Duchenne muscular dystrophy). - Sex-linked dominant diseases are extremely rare and affect both women and men who carry the defective X chromosome (e.g. a form of rickets) (JL, J, et Serre JL 1993).

- Multifactoriels diseases

Multifactoriels diseases appear to be the cause of the most common illnesses (cardiovasculardiseases, cancers, diabetes, neuropsychiatricdiseases)*("CCNE, Avis et recommandations sur " Génétique et médecine : de la prédiction à la prévention ", n°46, Rapport scientifique, 30 octobre 1995, Doc. Française, 1997, p. 38 et seq*.)

The latter are due to a genetic predisposition, often plurigenetic, resulting from the presence in the same individual of several genes whose interaction, or more simply cumulative action, favours the appearance of the disease under the influence of environmental factors.

However, genetic predisposition to a disease does not always correspond to a plurigenetic model. It may depend on a single gene. In fact, a disease linked to a monogenic predisposition is not monofactorial, since its onset is most often dependent on the interaction

of a genetic factor and external factors (JL, J, et Serre JL 1993). It is therefore multifactorial without being plurigenic.

Monogenic predispositions have been discovered mainly in the field of cancer. To detect plurigenetic or monogenic predispositions, researchers proceed either by analyzing phenotypic gene expression, or by genomic analysis ("CCNE, Avis et recommandationssur "Génétique et médecine : de la prédiction à la prévention ", n°46, Rapport scientifique, October 30, 1995, Doc. Française, 1997, p. 38 et seq.", n.d.).

At the beginning of the 20th century, the analysis of phenotypic gene expression was aimed at revealing correlations between blood groups and diseases. It showed that blood group A was slightly more frequent in stomach cancer patients than in controls (JL, J, et Serre JL 1993). Nonetheless, the association between disease and blood group, because it lacked precision, was not concrete (Ruffié 1993).

On the other hand, the discovery of the Major Histocompatibility System (HLA) by M. J. Dausset in the 1950s made it possible to rigorously define the risk factors presented by certain subjects (Ruffié 1993).

HLA antigens, which are the product of a dozen genes carried on chromosome 6, appear to be closely associated with many diseases: for example, HLA-B27 individuals are 87 times more likely to suffer from ankylosing spondylitis than other people. Similarly, in insulin-dependent diabetes, 90% of patients have DR3 and/or DR429 antigens. It soon became clear that most of these diseases were plurigenic, that HLA antigens were only a marker and that other genes were involved (Dausset 1992).

Researchers are no longer analyzing gene expression, but the genes themselves, in order to identify the sequence that governs gene expression. Born of an American initiative, the general project to sequence the entire human genome (Jordan 2001), launched in 1988, requires international cooperation between researchers. In France, for example, the Génoscoped'Evry, set up in 1997, has been entrusted with the total decoding of chromosome 14.

Deciphering the genome without knowing which parts correspond to genes would be a futile effort if, in parallel with sequencing, research were not carried out to identify the genes. The first step is to locate and isolate the region of the genome containing the gene. To do this, two different but complementary maps of the genome need to be drawn up. The first, the genetic

map, consists of tagging the genome with markers (genetic markers), indicating their positions in relation to each other. It calculates the genetic distance between two polymorphic points, i.e. DNA sequences that vary from one individual to another, by analyzing the frequency with which they are transmitted from parents to offspring. This tagging enables the approximate location of a disease gene between the various perfectly identified markers close to it. The physical map represents another approach to the genome, focusing on the direct study of DNA and aiming to calculate the effective distance, measured in DNA base pairs, between genes or markers. The physical map makes it possible to isolate the gene and know precisely which markers it lies between. Once the gene has been located and isolated, it is possible to identify the disease-causing mutation by comparing the gene sequence of healthy individuals with that of diseased individuals.

Through this genomic analysis, more than a dozen autosomal dominant single-gene predisposition genes have been identified (Thomas 1997). For example, an alteration in the BCRA1 (BReast Cancer 1) gene, located on chromosome 17, implies an 80% risk of developing breast cancer and a 40% risk of ovarian cancer.

Expressivity is variable in the sense that the existence of a cancer predisposition gene makes it impossible to predict which tumor might appear, when it will appear or even if it will occur (Feingold et al., n. d.).

In addition, there is genetic heterogeneity in cancer, meaning that different genes can cause the same syndromes (Feingold et al., n. d.). Only 5-10% of cancers are linked to single-gene predispositions. In fact, the majority of cancers involve plurigenetic predispositions. Because they are transmitted in a less systematic and more complex way, plurigenetic predispositions have been more difficult to study than monogenetic predispositions (Ruffié 1993). But efforts by researchers to determine the DNA differences between individuals should, through the mapping of genetic markers called SNPs (single nucleotide polymorphisms), make it possible to identify specific combinations that are at the root of most plurigenetic diseases. "For example, five genes are thought to be involved in diabetes, which affects around 10% of the population.

If one of us inherits the wrong SNP in four of these genes, we won't have a problem. But if it's present in all five, that person runs the risk of one day becoming diabetic. Whether the predisposition to the disease is monogenic or plurigenetic, its presence in the individual

indicates a latent state that does not make the onset of the disease certain, which is dependent on external causes. In this respect, disease predisposition genes differ from genes whose detection indicates a certainty of developing a future disease for which only the date of onset remains unknown (Huntington's disease). Uncertainty is linked to the incomplete penetrance of predisposition genes, by virtue of which the individual carrying the deleterious gene(s) will not necessarily develop the disease.

The gene is an often necessary, but always insufficient, cause of disease. This is not to say that the degree of uncertainty that affects the onset of genetic disease is identical depending on whether the individual carries a monogenic or plurigenetic predisposition. Indeed, whereas a monogenic predisposition gene alone creates a risk of contracting a disease, a plurigenetic predisposition gene is only likely to become pathogenic if it interacts with other susceptibility genes (Le Bihan et al. 1995).

4. Environmental mutagenesis

This is the process by which external agents, such as chemicals, radiation or biological factors, induce mutations in the DNA of living organisms. These mutations can alter the genetic material of cells and lead to changes in the phenotypic characteristics of organisms, sometimes with serious consequences for their health or survival. Mutagenic agents may be present in the natural environment, such as certain chemical compounds present in air, water or soil, or they may be of anthropogenic origin, resulting from human activity, such as industrial chemicals, pesticides, radioactive substances, etc.

The effects of environmental mutagenesis can be observed at different scales, from mutations at the molecular level to ecological consequences at the scale of populations and ecosystems. Consequently, understanding and monitoring environmental mutagenesis is crucial for assessing and mitigating risks to human health and the environment.

- Gene-environment interaction in the case of lung cancer

Advances in the field of genetic susceptibility to cancer are raising questions concerning the possible existence of interactions between exposure to carcinogensenvironmental and/or occupational carcinogens and the genetic polymorphisms involved in cancer susceptibility.

Bronchopulmonary cancers develop in a multi-stage process, characterized by progression towards an invasive phenotype of one or a small number of initial cells

by the acquisition of genetic alterations conferring a proliferative advantage (Hanahan and Weinberg, 2000). Many occupational and environmental carcinogens,
such as those found in tobacco smoke, can induce initiation of bronchial or alveolar and promote their progression.

These agents often affect the entire bronchopulmonary tree (as well as, in the case of tobacco smoke, the entire upper aero-digestive tract), and can independently initiate independently initiate distantly spaced cells, giving rise to several concomitant primary lesions. This phenomenon is described as field carcinogenesis.

The most common genetic alteration is mutation of the TP53 suppressor gene (chromosome 17p13). Its product, the p53 protein, is a transcription factor related to a family of proteins essential to epithelial differentiation and morphogenesis. a family of proteins essential for epithelial differentiation and morphogenesis, but specialized in the response to a broad spectrum of physical, chemical or biochemical stresses. P53 is an essential mediator of the cell response to exposure to carcinogens, capable of inducing cell cycle arrest, DNA repair or apoptosis depending on the cell type, degree of differentiation, nature and intensity of stress. Its role as a sensor of environmental changes makes it a key player in regulating the genetic and tissue stability of the bronchopulmonary epithelium.

TP53 mutations are mainly missense substitutions that inactivate the protein by preventing its folding into an active conformation protein by preventing its folding into an active conformation (Pfeifer et al., 2002). TP53 mutations are detected in 50% of NSCLC and over 70% of SCLC. In the SCC of heavy smokers, the frequency of mutations can exceed 80%. In smokers, mutations are found in both metaplasia and non-pathological epithelium.

The mutation, as it were, precedes tumour formation. On the other hand in the ADC of non-smoking women, the frequencies described in the literature vary between 25% and 50%, and it is thought that these mutations appear at a later stage of tumour progression. tumour progression. In smokers, the chemical nature of the mutation often constitutes a "molecular signature of mutagenic agents in tobacco smoke, such as benzo(a)pyrene and other polycyclic aromatic hydrocarbons (Le Calvez et al., 2005). In the cell exposed cell, these agents undergo bio-activation, generating metabolites capable of binding DNA covalently. The metabolites of benzo(a)pyrene bind guanines, and these same guanines are frequently mutated in smokers'

cancers. in smokers' cancers. This molecular "signature" is not present in the cancers of non-smokers.

5. Pharmacogénétique et pharmacogénomique

the response to medication varies from one subject to another and depends on environmental factors (diet, drug interaction, smoking), the patient's condition
(severity of disease, associated pathologies, age), therapeutic errors, but also genetic determinants. Pharmacogenetics is the study of the relationship between variability and therapeutic response. Its aim is to optimize therapeutic therapeutic decisions based on the individual's genome and the target molecule, which should improve patient care.
Genetics is now also essential for guiding cancer treatment with the study of the role of somatic mutations observed in tumors, and infectious diseases
infectious diseases by analyzing the genome of infectious agents. This branch of pharmacology came into its own over 50 years ago, when it was observed that acute hemolysis after taking an anti-malarial drug, primaquine, occurred in patients with hereditary glucose-6-phosphate dehydrogenase deficiency (Beutler 1993). Unlike pharmacogenetics, which studies the influence of genetic inheritance on drug pharmacogenomics studies the effects of drugs on the human genome. However, the two terms are often used interchangeably.
A drug is a xenobiotic, meaning a compound that is foreign to us. Its introduction into the body is followed by two stages, one of transformation, often in the liver, and a second of effect on the target, in variable order. These two stages are preceded by a phase of intestinal absorption for orally-administered drugs. All these steps are carried out by transporters and enzymes whose expression may vary according to the polymorphism of the gene concerned. In addition, the effect of the drug may also be influenced by variations in genes that are foreign to the metabolism and the target, and are involved in the body's response. After defining the concept of genetic polymorphism and describing genotyping techniques.

1. ***Genetic polymorphism***

An individual carries two alleles of the same gene, identical or different, defining the gene's constitutional state. There are two types of variation in these genes: repeat polymorphisms and single-nucleotide polymorphisms. The former, which are the most frequent, affect the number of tandem repeats of the same nucleotide sequence, also known as minisatellite (ten to fifteen nucleotides) or microsatellite (one to four nucleotides), depending on the extent of the repeat. This number varies from one individual to another and is inherited. Single nucleotide polymorphisms, or SNPs, are variations in the base of a nucleotide, with one replacing the other. Much more rarely, total or partial deletions of the gene lead to a defect in function, and amplifications lead to gains in function.

Polymorphisms in xenobiotic metabolism and transport enzymes enable us to distinguish, for the same drug, between slow metabolizers (lack of activity), fast metabolizers (generally normal activity) and ultra-fast metabolizers (excessive activity). In the definition of genetic polymorphism, a notion of frequency is added: the least frequent allele must be present in at least 1% of the population.

Routine identification of these polymorphisms relies on the polymerase chain reaction (PCR) and, more recently, on techniques that simultaneously analyze a large number of sites. Initially, dot blot and Southern blot were used, characterized respectively by the deposition or membrane transfer of denatured DNA fragments, followed by their recognition by hybridization to a labeled DNA probe of known nucleotide sequence. Currently, the DNA fragments carrying the polymorphism are most often amplified by PCR, then deposited on a membrane and recognized by hybridization to oligonucleotides specific to each allele.

The miniaturization of hybridization methods has also made it possible to examine thousands of nucleic acid molecules simultaneously on small-area solid matrices (Shi 2001). This makes it possible to search for unknown polymorphisms, or to determine which alleles of a known polymorphism are present in a given sample.

2. ***Variants of genes involved in drug metabolism and transport :*** Drug metabolism in the liver is carried out by two types of enzymes: phase I enzymes, such as cytochromes P450, which make molecules more polar and therefore more hydrophilic through hydroxylation, and phase II enzymes, which catalyze conjugation reactions with various radicals (glucuronate, sulfate, methyl, acetyl, glutathione...), thus reinforcing the hydrophilic character of drugs and their solubility in bile and urine. In addition to these enzymes, there are phase III transport proteins, such as the P-gp protein and proteins of the ABC (ATP binding cassette) family, which ensure the transfer of metabolites into or out of the cell ("JLE - Annales de Biologie Clinique - Médecine personnalisée, stratifiée, pharmacogénomique et biomarqueurs compagnons", n.d.).

Oxidative drug metabolism depends on first-phase enzymes, most of which belong to the same family of hemoproteins, cytochromes P450. In humans, some sixty genes have been identified, but only a small number of the twenty or so proteins encoded by these genes (CYP1, CYP2 and CYP3) contribute to drug metabolism (Wilkinson 2005).

They carry out around 80% of oxidation reactions, because their substrate specificity is relative and overlapping. As a result, drug or food interactions through substrate competition are frequent, and represent a major problem in dose optimization. These interactions may also involve other mechanisms, such as gene induction or repression. One of the most extensively studied polymorphisms concerns the CYP2D6 gene. Despite its low expression in the liver, the enzyme recognizes many drugs as substrates (β-blockers, tricyclic antidepressants, class I anti-arrhythmics, psychotropic drugs, etc.), representing a total of 25% of drugs used in everyday practice. The frequency of the different variants is far from negligible, and varies according to the population concerned. For example, CYP2D6*17 is found mainly in blacks (20-35% of the population), CYP2D6*10 is common in Southeast Asia (50% of the population) and CYP2D6*4 is not uncommon in Caucasians (12-21% of the population). These three variants are associated with reduced or zero activity and therefore slow metabolism, leading to overdoses (Ingelman-Sundberg 2005) or therapeutic inefficiencies if the enzyme converts a prodrug into an active drug (Gasche et al. 2004). On the other hand, CYP2D6*2xn (several copies of the gene), which leads to a gain of function, is found in

10-16% of Ethiopians and Saudi Arabians, and can lead to underdosing or the opposite if, here too, a prodrug is activated.

CYP2D6 has been studied mainly in the field of antidepressants and antipsychotics. Its variants associated with those of CYP2C19 explain many of the inappropriate responses to antidepressants and antipsychotics (Kirchheiner et al. 2004). It is advisable to adapt doses by decreasing them for carriers of gain-of-function mutations and increasing them for carriers of loss-of-function mutations. Such an approach would enable initial prescriptions to be fine-tuned without waiting for plasma drug concentrations to be monitored. Another polymorphism to consider is that of CYP2C9, several variants of which, accompanied by reduced activity, reach significant fractions of the white population: 20.4% for CYP2C9*1/*2 and 11.6% for CYP2C9*1/*3. This cytochrome recognizes about a hundred drugs as substrates, including anti-coagulants of the anti-vitamin K series, oral anti-diabetics, non-steroidal anti-inflammatory drugs and angiotensin II antagonists. This last class of drugs provides an example of the importance of knowing the metabolism and effects of a drug before assessing the consequences of genotyping. Indeed, the same CYP2C9 polymorphism leads to increased activity of losartan, whose metabolites are effective, and to increased inactivity of irbesartan, whose intact form alone is active.

The second-phase enzymes involved in metabolism are transferases, which couple the drug or its metabolite to a radical to facilitate elimination. Recognized polymorphisms generally have narrower distributions than cytochromes, but their consequences can be dramatic. One of the most extensively studied of these polymorphisms is that of thiopurine methyltransferase, which transfers a methyl radical to a metabolite of 6-mercaptopurine (Dervieux et al. 1999). There are three known mutations in the gene. Homozygotes (1/300 individuals) express no functional enzyme; 10% of the population is heterozygous and expresses intermediate levels of active enzyme; the remaining 90%, carriers of two wild-type alleles, have normal enzyme activity.

The absence of a functional enzyme in homozygotes leads to accumulation of 6-mercaptopurine, with the risk of severe aplasia. Heterozygotes can be treated, but with a reduced dosage. Another example of a transferase polymorphism is that of UDP-glucuronosyltransferase UGT1A1, an enzyme well known because its endogenous substrate

is bilirubin. The increase, from six to seven, of the TA tandem repeats in the "TATA box" sequence of the gene promoter is accompanied by a decrease in the enzyme's expression, characteristic of Gilbert's disease. This polymorphism is also involved in the biliary and urinary excretion of SN-38, the active metabolite of irinotecan which, like bilirubin, must be conjugated to a glucuronyl radical before being eliminated. SN-38 accumulates in patients with the mutation, leading to leukopenia and diarrhoea. Thus, depending on the genotype, a series of courses of chemotherapy may be prescribed with no foreseeable toxicity, or treatment may be discontinued (Ando et al. 2000).

Phase III excretion of drugs from the cell is ensured by the P-gp protein encoded by the MDR1 ("multidrug resistance") gene. By modulating plasma and intracellular concentrations, this protein influences the response to treatment with the drugs it carries, such as the antiproteases used in AIDS or immunosuppressants like tacrolimus. There is a mutation in exon 26 of the gene, with substitution of a thymine for a cytosine at position 3435 (3435C>T). The TT genotype is accompanied by an increase in plasma concentrations of these drugs, prompting consideration of dosage reduction (Hoffmeyer et al. 2000).

2.1. ***Somatic mutations of the target genome and drug response*** In the case of solid cancer tumors, mutations in the constitutional genome can, as we have seen above, modify the response to treatments such as 5-FU. But mutations in the tumor's somatic genome can also predict disease progression and response to treatment. We can either search for a mutation in a given gene, or analyze the entire transcriptome, revealing which genes are overexpressed, repressed or remain unchanged. All this adds up to the tumor's "identity card", so called because of its specificity. This identity card is an essential element in the therapeutic decision. In the case of head and neck cancers, for example, there is the problem of chemotherapy prior to surgery. When there is an inactivating mutation of the anti-tumor protein p53, chemotherapy is five times less likely to be effective than in the absence of mutation. Similarly, the detection of the estrogen receptor in breast cancer, whether or not associated with the progesterone receptor, leads to the use of anti-hormonal therapies such as

tamoxifen (Stearns, Davidson and Flockhart 2004), while the detection of the expression of the Her-2/neu oncogene leads to treatment with Herceptin®, an antibody directed against this oncogene (Khalili et al. 2005). In liposarcoma, co-amplification of the MDM2 and CD14 genes and a high degree of amplification of these genes are observed more frequently in dedifferentiated tumours than in well-differentiated tumours or in atypical lipomatous tumours. In the former case, resistance to chemotherapy and reduced survival are to be feared, whereas in the latter, a good response to chemotherapy and a favorable evolution can be expected (Hostein et al. 2004).

The example of mutations in the epidermal growth factor receptor (EGF-R) in lung cancer suggests treatment with IRESSA®, an inhibitor of this receptor's tyrosine kinase activity, or with a specific antibody (Khalili et al. 2005). Tumor genome analysis has been greatly facilitated by the creation of biological resource centers in healthcare establishments, the aim of which is to bring together samples from patients suffering from the same disease in a single structure, thereby enabling the influence of mutations on treatment efficacy to be assessed in a single study. The French National Academy of Medicine has published a report on this subject (Mukohara et al. 2005).

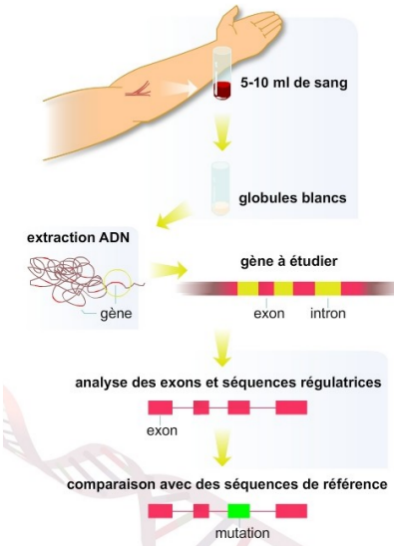
3. ***Phenotyping and genotyping :***

Phenotyping is possible when the variation sought concerns an enzyme activity. It is usually carried out in vivo. The subject ingests a dose of the drug, generally lower than that recommended for therapeutic use. Blood and/or urine samples are then collected at times chosen according to the product's pharmacokinetics.

In these samples, the intact and metabolized forms of the drug are measured, usually by chromatography. The metabolite-to-substrate ratio is then calculated, making it possible to separate ultra-fast, fast, intermediate and slow metabolizers by comparing the results with those obtained on a large population. Phenotyping is a time-consuming, demanding and costly method, requiring the administration of a test drug. However, it is indispensable in the first instance. In fact, phenotyping is essential for a precise understanding of drug metabolism, enabling us to identify candidate genes.

If the study of genes precedes that of variations in therapeutic efficacy, phenotyping remains essential to validate the genotyping data. When drug administration to healthy subjects is contraindicated due to toxicity (anti-tumor or immunosuppressive agents, for example), in vitro phenotyping can be attempted. This assumes - a condition rarely met - that the enzyme under study is present in easily accessible tissues such as blood cells.

6. **Molecular diagnostics in oncogenetics**



Molecular diagnosis in oncogenetics involves identifying individuals at risk by identifying specific genetic alterations associated with the development of cancer in an individual. These alterations may include gene mutations, gene amplifications or deletions, chromosomal rearrangements, or epigenetic modifications, which can play a crucial role in cancer initiation, progression and response to treatment. Gene testing is based on clinical arguments and family history. The identification of molecular alterations in cancer cells has led not only to a better understanding of the mechanisms involved in the development of the disease, but also to the development of therapies targeting these molecular anomalies.

Molecular characterization of the tumor has thus become a decisive criterion in the choice of therapeutic strategy. It makes it possible to restrict the prescription of a certain number of treatments to those patients likely to benefit from them. The deployment of next-generation sequencing (NGS) has enabled the analysis of multiple molecular alterations constituting potential therapeutic targets. For the analysis of solid tumors, a minimum list of 16 genes has been published by INCa1 for routine use. DNA NGS (DNAseq) is not always sufficient to analyze all the biomarkers required, and complementary techniques are deployed in clinical practice, such as immunohistochemistry (IHC) to study the expression of proteins like PD-L1 and ALK, or RNA NGS sequencing (RNAseq) to search for fusion transcripts. Moreover, while the search for these molecular anomalies is still classically carried out on formalin-fixed, paraffin-embedded tumor tissue, an alternative biological source, generically referred to as "liquid biopsy", is increasingly being used.

This term refers to the isolation of biological material released by the tumor in body fluids, most often blood, but also cerebrospinal fluid in the case of brain metastases, and pleural fluid in the case of metastatic pleurisy. With a few technical limitations, analysis of this biological material provides less invasive, real-time access to certain tumour molecular abnormalities, and can be repeated during the course of the patient's treatment, thus reducing the number of unnecessary, toxic and costly treatments.

6. Main mutation detection methods

A mutation is any modification affecting the genetic material or genome of a cell, containing the hereditary message. We can approach these modifications from two distinct points of view: on the one hand, mutations that occur spontaneously in nature, whether or not they are passed on to subsequent generations; on the other, we can consider the possibility of inducing these mutations ourselves. Several aspects concerning mutations are essential from the scientist's point of view. Firstly, they can lead to the total loss of information and the deletion of the protein encoded by the mutated gene, or else to an alteration in the structure of this protein. An altered protein represents a non-functional molecule, and this dysfunction is of paramount importance in molecular diagnostics, as it may be at the root of most pathologies. There are also mutations which do not radically affect the structure or function of the protein concerned, but which are nonetheless varied and widespread within populations. These are often variations in a single nucleotide, known as SNPs (Single Nucleotide Polymorphism).

From an epidemiological and clinical point of view, we must make the following distinctions:

- There's a big difference between so-called essential mutations (affecting the entirety/ functionality of the protein) and non-essential mutations (often occurring in non-coding, non-regulatory regions of the DNA). Clearly, our interest lies in the former of these two categories.
- A distinction is made between point mutations, affecting a single nitrogenous base (deletions, insertions, missense or nonsense mutations), and broad mutations, affecting up to entire chromosomal regions (deletions, insertions, inversions, repeats, translocations); both categories are of interest to the clinician.
- A clear distinction must be made between somatic and germline mutations. If a mutation occurs in a germline cell (part of the set of cells that produce gametes), it will be transmitted

to the offspring of these cells. The mutation is then hereditary. Most of these mutations prevent the egg cell from developing. If the egg is able to develop, the resulting individual suffers from various malformations. If a mutation occurs in a somatic cell (not part of the germ line), it cannot be passed on to a descendant - it is not hereditary. While somatic mutations are of great interest for gene therapy in oncology, germline mutations are of interest for molecular epidemiology, statistics and, above all, human medical genetics.

- Finally, and this is the starting point for the choice of detection methods, for each gene there are known mutations, repetitive from one individual to another and stored in international databases, but above all a huge number of unknown mutations, appearing de novo or characteristic of a single family line or a single individual. For the detection of the latter, the combination of several detection techniques is absolutely essential. The detection of DNA mutations is an essential step in molecular biology, for both basic research and medical applications. The strategies currently available to detect mutated alleles are based either on the use of rapid methods limited to the identification of a small number of specific mutants, or on the use of costly and laborious techniques capable of detecting the slightest sequence alteration in the essential portions of genes.

Example of some mutation detection methods and their principles

- Sanger sequencing

The search for an **unknown mutation** by Sanger sequencing is considered a qualitative method: the aim is to detect the presence of a qualitative sequence variation in relation to a reference sequence.

The sequence reaction itself involves in vitro synthesis of DNA from a template with random incorporation of dideoxynucleotide triphosphate (ddNTP). The template (previously purified PCR products) must be present in large quantities (> 100 million copies in the reaction tube, hence the need for the first PCR amplification step). It is added to a reaction mixture comprising a DNA polymerase (Taq polymerase), a primer hybridizing 5' to the fragment to be sequenced, a buffer and a mixture of dNTP (deoxynucleotide triphosphate) +ddNTP. Each ddNTP is labelled with a specific fluorochrome. The ddNTP/dNTP ratio is of the order of 1:100. There is therefore a 100-fold greater chance of incorporating a dNTP than a ddNTP.

Incorporation of a ddNTP stops the elongation reaction. DNA synthesis is complementary to the initial template. Thus, at each position of A on the template strand, a fluorescent dTTP or ddTTP will be incorporated. As the sequence reaction is carried out on a very large number of copies of the template, random incorporation of the ddNTPs leads to their incorporation at every possible position on the DNA segment. Thus, for a 400 bp PCR product, 400 types of fluorescent fragments will be generated, differing in size by only one base and in the nature of the ddNTP incorporated at their end. These neosynthesized fragments are then separated by migration into the capillaries of the automatic sequencer. A laser beam directed at the capillaries excites the ddNTP fluorochrome. A CCD camera captures the fluorescence emission amplified by a photomultiplier. An electropherogram is obtained after analysis of the raw data returned by the CCD camera using analysis software (background correction, base recognition, calculation of Phred score or equivalent quality value). The patient's electropherogram is then compared with that of a control subject, or with a reference sequence, in order to identify a homozygous (or hemizygous, depending on the mode of transmission of the disease in question) or heterozygous sequence variation by modifying the profile.

- Taqman probe

Allelic discrimination using Taqman® fluorescent probes involves the use of two fluorescent probes differing by just one nucleotide, and complementary to either the wild-type or mutated allele. This technique produces a qualitative result (presence/absence of a mutation).
The quantitative PCR technique using Taqman® probes is based on real-time PCR using specific oligonucleotide probes labeled with two fluorophores, one of which is quenched by a so-called "reporter". Degradation of this probe by Taq polymerase during each PCR cycle is accompanied by an increase in Reporter fluorescence, which is measured. The use of this technique for allelic discrimination involves hybridizing two fluorescent probes that differ by just one nucleotide and are complementary to either the wild-type or mutated allele of interest. Only correctly hybridized probes will be degraded by Taq polymerase and emit a fluorescent signal, enabling a mutated allele to be identified and quantified in relation to the wild-type allele.

- Detection of a known mutation by the pcr-multiplex

When we know exactly the nature and location of a certain mutation to be detected, we can target the relevant region of the genome, or even the gene of interest, using a simple and highly efficient technique. We won't dwell here on the detailed description of PCR (Polymerase Chain Reaction).

The principle of multiplex PCR is simple: a set of several primer pairs is used to simultaneously amplify several DNA targets in a single PCR reaction, rather than a single primer pair to amplify a single DNA target. In practice, multiplex PCR is not that simple. Set-up is often tedious, because as the number of targets to be amplified in a single reaction increases, so does the level of complexity. Optimization is usually required before the full benefits of multiplex PCR can be realized.

Chapter III

Genome instability
Cancer inflammation

I. Cancer cell metabolism

1. Warburg effect

In 1956, Otto Warburg published an article in which he made the following observation: mouse ascites cancer cells consume 7 mm3 O2.mg-1.h-1 and produce 60 mm3 .mg-1 .h-1 lactic acid by fermentation. By converting these data into energy equivalents, Warburg shows that cancer cells produce as much energy through fermentation, i.e. the conversion of pyruvate from glycolysis into lactate, as through respiration, whereas healthy cells produce more energy through respiration than through fermentation. He then put forward the idea that cancer cells exhibit exacerbated glycolytic activity in response to increased energy requirements, even in the presence of O2, enabling them to proliferate intensively (Warburg O, 1956). This theory became known as "aerobic glycolysis" or the "Warburg effect". According to Warburg, the reason why cancer cells produce so little energy through mitochondrial respiration is that the latter is irreversibly altered as a result of a reduction in the cell's O2 consumption, or a break between ATP formation and mitochondrial respiration. Furthermore, he cites the use of arsenic, a poison specific to the mitochondrial respiratory chain, as being capable of inducing cancer (Warburg O, 1956). However, as early as 1957, Weinhouse's team demonstrated that the mitochondria of tumor cells remained functional. They showed that tumors grafted into mice were able to take up carbon-14-labelled fatty acids and incorporate them in vivo, and that cancer cells could use fatty acids for respiration in vitro (Medes G, Paden G and Weinhouse S, 1957). Numerous studies have now shown that, despite intense glycolytic activity, even in the presence of O2, cancer cell mitochondria remain functional and can actively participate in tumorigenesis (reviewed by Porporato PE et al., 2018). Although mutations in Krebs cycle enzymes do not predispose to cancer, isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH) or fumarate hydratase (FH) are exceptions, since mutations in these enzymes have been implicated in the development of acute leukemia (Rakheja D et al., 2012), paranglioma and renal cell carcinoma respectively (King A et al., 2006). In untransformed, differentiated cells with a low level of cell division, cells metabolize glucose into CO2 and H2O through glycolysis and the Krebs cycle, in order to generate ATP via OXPHOS. However, healthy cells sometimes need to increase their cell division rapidly, as in immune responses to antigens, tissue regeneration or embryonic development. Cancer cells also share these characteristics. To meet their energy and anabolic

requirements for proliferation, cancer cells must synthesize lipids for membranes, amino acids to form proteins and nucleic acids for DNA. To achieve this, cancer cells must prefer anabolic pathways. Glycolysis metabolizes glucose to form the intermediates G6P, F6P and G3P, and can supply macromolecules via the pentose phosphate and ribose-5-P pathways. 3-PG can produce AA via the serine pathway and the transamination of pyruvate to alanine. G3P and 3-PG can also participate in the synthesis of phospholipids for membrane manufacture (Lunt SY and Vander Heiden MG, 2011).

- **<u>Molecular mechanisms favoring the warburg effect :</u>**

ancer cells exhibit enhanced glucose uptake and metabolism through aerobic glycolysis, in part due to the overexpression of c-myc (Miller DM et al., 2012) and HIF1a (Courtnay R et al., 2015), which in turn stimulate glycolytic enzymes such as hexokinase II, lactate dehydrogenase, pyruvate kinase and pyruvate dehydrogenase kinase. These enzymes direct pyruvate towards lactate formation and reduce its entry into the mitochondria. The expression of numerous glycolytic enzymes is thus altered, contributing to tumorigenesis.

Indeed, cancer cells reduce pyruvate entry into mitochondria, which in turn reduces pyruvate conversion to acetyl-coA and slows down the Krebs cycle. Among the mechanisms responsible for the decrease in pyruvate entry into mitochondria, increased expression of LDH-A (Feng Y et al., 2018) and decreased expression of the M2 isoform of pyruvate kinase (PKM2) (Dayton TL et al., 2016) have been shown to be involved in the induction of the Warburg effect. Decreased PKM2 expression redirects glycolytic intermediates to branched pathways of glycolysis, such as the pentose phosphate pathway to promote NADPH production, and the serine synthesis pathway (Dayton TL et al., 2016)

<u>2. Involvement of pyruvate metabolism in the Warburg phenotype</u>

- ***Pyruvate kinase***

Although numerous mechanisms are involved in metabolic changes in cancer cells, pyruvate synthesis and metabolism play a central role in these changes. The 10th and final step of glycolysis is catalyzed by pyruvate kinase, a phosphotransferase catalyzing the reaction PEP + ADP à pyruvate + ATP. In mammals, this enzyme is encoded by 2 genes, each of which can give rise to 2 isoforms: the PKLR gene codes for the PKR and PKL isoforms, and the PKM gene codes for the PKM1 and PKM2 isoforms. The PKR enyme is expressed in red blood

cells, while PKL is expressed in the liver and certain cells of the pancreas, intestines and kidneys. The PKM1 isoform is a constitutively active tetrameric enzyme expressed in ATP-intensive differentiated adult tissues such as heart, muscle and brain. The PKM2 isoform, on the other hand, is mainly expressed during embryogenesis, regeneration and in adult tissues such as spleen and lung, where its activity is very high in actively proliferating cells (Tsutsumi H et al., 1988). To date, most tumors studied, such as prostate cancer (Wong N et al., 2014) or colorectal cancer (Zhou CF et al., 2012), have shown PKM2 overexpression under the control of the c-Myc oncoprotein (Kim JW et al., 2004), suggesting a selective advantage for this isoform. Indeed, a meta-analysis study of 25 scientific publications showed that PKM2 expression correlated with poor prognosis in and unfavorable overall survival in breast cancer, squamous cell carcinoma of the esophagus, hepatocellular carcinoma and bladder cancer, and poor overall survival in pancreatic and gastric cancers (Zhu H et al., 2017).Some authors have hypothesized that the level of PKM2 enzymatic activity could be an adaptive response to the cell's different metabolic needs: during cell proliferation, PKM2 activity decreases, whereas during the phases of tumor initiation and progression, its activity increases. In particular, when the tumor forms at a primary or metastatic site, it must be able to grow in a different, or even inappropriate, microenvironment, and cope with poor vascularization or nutrient stress, which can limit its growth. Cancer cells therefore require an adapted metabolic program enabling them to efficiently synthesize ATP at the expense of anabolic metabolism, and therefore redirect pyruvate more at the OXPHOS level, which would explain their need for greater pyruvate kinase activity (Dong G et al., 2016). PKM2 mutations observed in human tumors are heterozygous: as such, a mutation reducing PKM2 activity would be conducive to proliferative metabolism, although cells retain a functional PKM allele allowing them metabolic flexibility to adapt to different stresses (Israelsen WJ et al., 2013) (Dayton TL et al., 2016). PKM2 also plays a major role in maintaining the metabolic program of cancer cells. Authors have shown that inhibiting PKM2 expression using short hairpin RNA (shRNA) and replacing it with PKM1 expression reversed the Warburg effect: cancer cells showed an increase in O2 consumption at the expense of a reduction in lactate production (i.e. aerobic glycolysis), as well as a decrease in their ability to form tumors in vivo after xenotransplantation. These results therefore demonstrate the major role of PKM2 in aerobic glycolysis and metabolic phenotype, giving cancer cells a

selective advantage in tumor growth in vivo (Christofk HR et al., 2008). Furthermore, inhibition of PKM2 expression promotes the redirection of metabolites from glycolysis to upstream branched pathways such as the pentose phosphate pathway (Mullarky E and Cantley LC, 2015).

- ***The PDK/PDH axis***

One consequence of the conversion of pyruvate to lactate is the impairment of glucose-derived pyruvate oxidation, inducing an uncoupling of glycolysis from Krebs cycle functions (Marchetti P et al., 2014). This mechanism involves PDKs, which phosphorylate and deactivate PDH. In many cancers, such as breast cancer, colon cancer, gastric cancer, hepatocellular carcinoma or melanoma, overexpression of PDKs is associated with poor prognosis and decreased overall survival in patients (Zhang W et al., Sci 2015). PDK plays a major role in cancer cell metabolism, since inhibition of PDKs and thus activation of PDH increases OXPHOS, which could be a strategy to reverse the Warburg effect and limit cancer cell proliferation (Zhang W et al., Sci 2015). Our team has shown in a metastatic melanoma model that these cells exhibit decreased OXPHOS activity in association with elevated nuclear expression of HIF-1a (Kluza J et al., 2012). In fact, inhibition of this factor induces a switch from glycolysis to mitochondrial respiration thanks to reduced expression of the PDK3 kinase, which can no longer inhibit PDH (Kluza J et al., 2012). PDH catalyzes the conversion of pyruvate to acetyl-CoA, and its activity can be inhibited by phosphorylation by PDK. High PDK activity, low PDH activity and dependence on glycolysis are often correlated with high tumorigenicity. In many types of cancer such as breast cancer, colon cancer, melanoma, or myeloma, PDK expression is increased and is often associated with a poor prognosis in patients (reviewed by Zhang W et al., Sci 2015). In the case of prostate cancer, authors have shown that resveratrol induces metabolic reprogramming in cancer cells. By targeting PDH, a key enzyme in mitochondrial energy metabolism, resveratrol increases glucose and pyruvate oxidation within the mitochondria, and decreases lactate production, thus reversing the Warburg effect. This polyphenol, naturally present in certain foods, on the one hand increased the oxidative capacity of cancer cells, and on the other decreased their glycolytic activity and the PP pathway, thus inducing tumor growth inhibition (Saunier E et al., 2017).

- ***Lactate dehydrogenase***

Lactate dehydrogenase (LDH) is an enzyme that reduces pyruvate to lactate and recycles NAD+ , which is then recycled to maintain sustained glycolytic activity. In cancer, LDH-A and MCT-1/4 transporters, which convert pyruvate to lactate and export lactate respectively, are often overexpressed, leading to reduced pyruvate oxidation in mitochondria (Higashi K et al., 2000) (Park SJ et al., 2018).

- ***HIF-1α***

HIF (Hypoxia-inducible factor) is a heterodimeric transcription factor, composed of two subunits:HIF-1β and HIF-1α. HIF-1β is constitutively expressed, while HIF-1α expression is regulated by O2 content. In normoxia, i.e. in the presence of O2, HIF-1α is rapidly degraded by hydroxylation of residues 402 and 564 by proline hydroxylase (PHD), which are then recognized by the Von Hippel-Lindau protein (pVHL), which in turn is recognized by the E3 ubiquitin ligase protein (Sadri N and Zhang P, 2013). Conversely, when O2 tension falls sharply, i.e. in hypoxia, the subunit is no longer hydroxylated and is therefore stabilized. HIF-1α can also be activated by numerous factors, such as growth factors, loss of tumor suppressors, oncogenic activation or mutation of mitochondrial enzymes. This activated transcription factor is translocated to the nucleus, where it heterodimerizes with HIF-1β and recruits co-activator proteins (p300-CBP). This complex will then bind to the hypoxia response element and induce transcription of target genes involved in angiogenesis (VEGF), metabolic reprogramming (GLUT, PDK1), survival (anti-apoptotic protein IGF-1 and IGF-2), treatment resistance (ABCB1), staining properties (DLK1) as well as invasion and metastasis (Wigerup C et al., 2016). In melanoma, aberrant expression ofHIF-1α in normoxia leads to mechanisms such as MITF mutation, aberrant activation of the MEK/ERK signaling pathway particularly in melanomas with BRAFV600E or Ras mutations or activation of the PI3K pathway (reviewed by Marchetti P et al., 2014).

Hypoxia through HIF is also involved in changing the metabolism of cancer cells. Stabilization of HIF1, HIF2 and p53, as well as Myc expression, lead to gradual metabolic changes in response to increased hypoxia, such as activation of genes involved in glucose transport (GLUT1 and GLUT3) and glycolysis (HK2, MCT4, LDHA, PDK1), thereby promoting glycolytic activity and inhibiting mitochondrial OXPHOS (Eales KL et al., 2016).

II. Acidosis: a consequence of the Warburg effect

Due to their high glycolytic activity, and hence the Warburg effect, cancer cells secrete large quantities of lactate and H+, which accumulate in their microenvironment, resulting in a decrease in the pH of the cellular environment and the extracellular matrix (ECM). This acidosis is also induced by hypoxia, which by stabilizing the transcription factor HIF-1α (hypoxia-inducible factor 1α) enables the activation of genes involved in glucose transport such as GLUT-1 (Chen C et al., 2001), as well as glycolytic enzymes such as aldolase-A, PGK-1 and LDH-A (Semenza GL et al., 1994).

Hypoxia in tumors can also induce a metabolic shift from OXPHOS to glycolysis, leading to acidosis. In cancer cells, the HIF factor enables them to adapt to low O2 tension. The resulting acidosis also stabilizes HIF-1α and regulates HIF-induced genes (Chiche J et al., 2010). This pH change can Induce extracellular matrix remodeling and promote cancer cell migration, invasion and metastasis, but also angiogenesis and blood vessel invasion in response to angiogenic factors produced at tumor level (reviewed by Thews O and Riemann A, 2019). Authors have shown that lactate can stabilize HIF-1α and increase VEGF (vascular endothelial growth factor) expression in aerobic human endothelial cell cultures, and that inhibition of LDH by oxamate prevents lactate-induced angiogenic effects (Hunt TK et al., 2007). In the case of highly glycolytic melanoma cells, the expression of GLUT-1 and MCT-4 transporters, allowing glucose import and lactate export respectively, was shown to be increased in primary patient metastasis samples (Pinheiro C et al., 2016). Furthermore, the increase in lactate secretion in the microenvironment induced by HIF-1α and Myc, participates in the alteration of the tumor microenvironment, promoting angiogenesis, metastasis and immune suppression (reviewed by Romero-Garcia S et al., 2016). Acidosis can also induce reprogramming towards other metabolic pathways. In the case of breast cancer, acidosis allows glucose to be directed more towards the oxidative branch of the pentose phosphate pathway, thereby increasing NADPH production and recycling the GSH pool to combat the oxidative stress induced when pH is very low.Reduced pH can also reprogram the cellular metabolism of cancer cells, favoring the use of glutaminolysis and β−oxidation. To this end, under low pH, the G6PD and GLS2 genes are induced to disconnect ribose production from the oxidative pentose phosphate pathway, redirecting

metabolites towards glutaminolysis (LaMonte G et al., 2013). Unlike normal cells, which die when extracellular pH falls sharply, cancer cells are equipped to resist this acidosis. To cope with it, they increase the expression of transporters and exchangers exporting H+ protons, such as monocarboxylate transporters (MCTs) transporting lactate bidirectionally, Na+ /H+ exchangers, proton pumps, bicarbonate (HCO3-) transporters and H+ and HCO3 - exchangers (reviewed by Corbet C and Feron O, 2017).

Acidosis also plays a part in resistance to anti-cancer treatments in certain cancers. In BRAFV600E-mutated melanoma, for example, authors have shown that acute or chronic exposure to an acidic microenvironment causes cancer cells to undergo epithelial-mesenchymal transition-like adaptation, with reduced proliferation and high resistance to apoptosis. In contrast to cells grown in medium at standard pH, cells exposed to more acidic medium are resistant to treatment with Vemurafenib and Trametinib (Ruzzolini J et al., 2017).

Lactate can also serve as a substrate for cancer cells. In human non-small-cell lung cancer, cells can oxidize glucose through the Krebs cycle. However, the most aggressive and glucose-intensive cells are also able to take up lactate via the MCT-1 transporter and use it as a substrate, both in vitro and in vivo (Faubert B et al., 2017). Anticancer treatments can also be responsible for acidosis in cancer cells. In the HeLa cervical cancer cell line, cisplatin chemotherapy treatment induces cytoplasmic acidification shortly after cell treatment. The authors show that cancer cells are able to set up a system enabling them to maintain an alkaline pH.

However, after prolonged treatments in vitro and in vivo, acidification of the intracellular pH induces a metabolic shift in cells from glycolysis to OXPHOS, resulting in inhibition of cancer cell growth (Shirmanova MV et al., 2017). In conclusion, targeting lactate import and export, by targeting MCT transporters, could be effective in affecting cancer cell survival and growth. Authors have shown in a squamous cervical cancer model that inhibition of lactate export by inhibiting the MCT-1 transporter with α-cyano-4-hydroxycinnamate (CHC) was effective in reducing tumor growth in vivo in xenografted mice (Sonveaux P et al., 2008).

III. The role of mitochondrial metabolism in cancer cells:

Despite the Warburg effect, mitochondrial metabolism has been shown to be involved in cancer progression and resistance to anti-cancer treatments. The mitochondria represent a major source of ATP and anabolic precursors required for cancer cell proliferation. Through the synthesis of mitochondrial ROS, mitochondria also participate in cancer cell mutagenesis and diversification (Porporato PE et al., 2018).

The role of substrates in supplying mitochondrial OXPHOS:

Deregulation of mitochondrial pyruvate transport in cancer:

MPC has been shown to participate in tumor initiation and progression (Schell RJ and Rutter J, 2013). Furthermore, under-expression of this transporter in patients is often correlated with a poor prognosis. In prostate cancer, MPC1 and MPC2 transporter expression is associated with a favorable prognosis in prostate cancer patients (Li X et al., 2016) and in lung adenocarcinoma (ACP). In another hormone-dependent prostate cancer model, MPC expression is controlled by androgen receptors. Inhibition of pyruvate import inhibits cell proliferation and metabolic pathways associated with the Krebs cycle, such as lipogenesis and OXPHOS.

In summary, pyruvate import into mitochondria could constitute a therapeutically targetable metabolic vulnerability to affect the survival and proliferation of hormone-dependent prostate cancer cells (Badere DA et al., 2019). In addition to its role in metabolism, MPC1 also possesses a role in suppressing strain, invasion and migration of PCA cancer cells through the MPC1/STAT3 axis: by interacting with mito-STAT3 (mitochondrial signal transducer and activator of transcription 3), the MPC-1 transporter decreases its phosphorylation and translocation into the nucleus, which then induces inhibition of genes involved in the aforementioned tumorigenic mechanisms (Zou H et al., 2019). In a colon cancer model, authors showed that re-expression of MPC1 and MPC2 transporters increased pyruvate oxidation in mitochondria, and impaired colony and spheroid formation in vitro, as well as xenograft growth in vivo. Furthermore, they show in this model that pyruvate transport is also involved in the maintenance and fate of colon cancer stem cells (Schell JC et al., 2014).

Deregulation of glutamine metabolism in cancer

Numerous studies have now demonstrated the importance of glutamine as an essential bioenergetic and anabolic substrate in various types of cancer, such as acute myeloid leukemia (Willems L et al., 2013), melanoma (Hernandez-Davies JE et al., 2015), breast cancer (Demas DM et al., 2019) or prostate cancer (Zacharias NM et al., 2017), in order to meet their demands for ATP, biosynthetic precursors and reducing agents. Indeed, cancer cells are able to use glutamine as a source of carbons through the Krebs cycle to fuel other biosynthetic pathways. Glutamine is also used as a nitrogen donor and transporter, as an exchanger for the import of other amino acids (AA), and as a signaling molecule. It also plays a role in the control of redox potential through the synthesis of NADPH (Bott A et al., 2019).

Glutamine is involved in the import of leucine into cells, and this contributes to the control of signalling associating amino acids/rag and mTORC1. Authors have shown that glutamine depletion induced by the L-Asparaginases Kidrolase and Erwinase induces mTORC1 inhibition and enhances cell death in numerous AML lines (Willems L et al., 2013).

1.1.1.1. Glutamine transport

In order to meet the high demand for glutamine, cancer cells use glutamine-specific transporters with a high transport capacity. Glutamine can be recognised by certain members of the SLC (solute carrier) family of transporters such as SLC1, SLC6, SLC7 and SLC38 (Pochini L et al., 2014).

This AA is taken up in a Na+ -dependent manner by the amino acid transporter via the Alanine-Serine-Cysteine transporter type 2 (ASCT2, encoded by the SLC1A5 gene) located on the plasma membrane, in exchange for other neutral AAs such as serine, asparagine or threonine. Other Na+ -dependent transporters of the SLC6 family, such as ATB0,+ and B0AT1, encoded by the SLC6A14 and SLC6A19 genes respectively, also have specificity for glutamine and possess high transport capacities, thus making it possible to supply cells under both physiological and pathological conditions (Pramod AB et al., 2013).

The ASCT2 transporter is widely expressed in many tissues, such as lung, skeletal muscle, kidney, large intestine, brain, adipose tissue (Kanai Y et al., 2013), but SLC1A5 expression is also often increased in many human cancers, such as prostate, breast, ovarian, kidney, stomach, hepatocarcinoma, neuroblastoma, etc. (for review: Scalise M et al., 2017). Authors have shown that glutamine influx via ASCT2 induces AA influx via the L-type AA

transporter 1 (LAT1) exchanger, which activates the mTORC1 (mechanistic target of rapamycin complex 1) protein and stimulates cell growth. These 2 transporters have been described as having a pro-tumour role, and an increase in their expression is correlated with a poor prognosis in patients with numerous types of cancer, such as non-small cell lung cancer, prostate cancer and colorectal cancer (Hassanein M et al., 2013) (Sakata T et al., 2009) (Huang F et al., 2014).

ASCT2 knockout has been shown to decrease glutamine import by more than 60% in lung and colon adenocarcinoma cell lines, thereby reducing cell growth without inducing a stress response to AA depletion or altering mTORC1 activity (Cormerais Y et al., 2017).

1.1.1.1 Anaplerotic reactions from glutamine

The carboxylation of pyruvate to oxaloacetate and the synthesis of glutamate from glutamine are the two anaplerotic biochemical reactions that supply intermediates to the Krebs cycle. Glutamine is a 5-carbon AA. After entering the cell, glutamine is converted into glutamate by the glutaminase GLS1 in the mitochondria. Glutamate can be (1) converted to asparatate for nucleoside synthesis or (2) converted to a-KG by glutamate dehydrogenase (GDH) or by transaminases from alanine (ALT) or aspartate (AST), which transfer the amino group from glutamate to a keto acid, which enters the Krebs cycle in the mitochondria. The a-KG undergoes successive reactions to produce malate and ATP via succinyl-CoA synthetase. This process releases one carbon atom in the form of CO2. The other 4 carbons are used to produce malate, which can leave the mitochondria, where it is converted into pyruvate, generating NADPH for the production of fatty acids or for biosynthetic pathways.

The pyruvate resulting from these reactions can be used to produce glucose, a phenomenon known as "reverse glycolysis", to re-enter the PPP to increase the production of NADPH, or be transformed into lactate to regenerate NAD+ which can be used for anaerobic glycolysis and the production of ATP.

Citrate in turn is catalysed (1) to isocitrate, then to a-KG, generating another NADPH molecule or (2) to ACoA for fatty acid synthesis and AOA for non-essential AA synthesis (Scalise M et al., 2017). Increased glutamine uptake allows cancer cells to synthesise macromolecules required for proliferation, but these events require high energy consumption, which is provided through increased glucose and glutamine uptake, increased expression of metabolic enzymes and their enzymatic activity. Glutaminase overexpression induced by c-

Myc is often observed in many types of cancer such as glioma (Wise DR et al., 2008), multiple myeloma (Effenbergr M et al., 2017) and colorectal cancer (Song Z et al., 2017).

Craig B. Thompson showed that proliferating glioblastoma cells exhibit aerobic glycolysis and an active Krebs cycle characterised by efflux of substrates for biosynthetic pathways, thanks to NADPH and anaplerosis. To produce GAs, they use glucose as a lipogenic substrate. However, GA synthesis requires NADPH and a mechanism to feed the Krebs cycle. To this end, glioblastoma cells increase their uptake of glutamine, which they convert into lactate in order to rapidly produce NADPH.

They can also use glutamine, which they convert into malate and then AOA, to feed the Krebs cycle. In this model, therefore, glutamine is not used to supply nitrogen for nucleotide synthesis or the maintenance of non-essential AAs, but contributes as a carbon source to biosynthetic pathways (DeBeraradinis RJ et al., 2007). Cells can also produce other AAs, such as asparagine, by converting glutamine to malate and then to aspartate and asparagine via malate dehydrogenase and aspartate aminotranferase respectively.

VI . Targeting glutamine metabolism in cancer

Numerous studies to date have shown that glutamine metabolism plays a major role in the process of cell tumorigenesis. Because of the complex network of enzymes and transporters involved in glutamine metabolism, a number of compounds have been developed to target glutamine transport, such as tamoxifen or L- γ-glutamyl-pnitroanilide (GPNA) targeting ASCT, or to target glutaminolysis enzymes, such as CB-839 and BPTES targeting GLS1. Some molecules target glutamine directly, such as L-Asparaginase, which converts glutamine into glutamate to prevent it from entering the cell (Chen L and Cui H, 2015).

Prof. Bouscary's team showed in acute myeloid leukaemia that glutamine controlled mitochondrial OXPHOS, and that targeting glutaminolysis was effective in affecting the survival of leukaemia cells. Inhibition of GLS1 glutaminase by knockdown or by the compound CB-839 induces a decrease in OXPHOS activity, resulting in cell proliferation arrest and induction of apoptosis of leukaemic cells in vitro, as well as inhibition of AML development in vivo in NOD-Scid-Gamma (NSG) mice, without affecting healthy CD34+ cells (Jacque N et al., 2015). Another study showed that in AML, a subtype of cells exhibited increased expression of GLS1 encoding two isoforms: renal (KGA) and glutaminase C

(GAC), as well as increased sensitivity to CB-839-induced glutamine deprivation (Matre P et al., 2016).

- ***Deregulation of fatty acid synthesis***

Although untransformed human cells prefer to take up exogenous sources of lipids, cancer cells prefer de novo lipid synthesis (Medes G et al., 1953), to support processes such as lipid membrane formation and signalling. Citrate, an essential intermediate in the formation of fatty acids, can be obtained from glucose followed by the Krebs cycle, or from glutamine by glutaminolysis followed by reductive carboxylation. Cancer cells oxidise glucose and incorporate its carbons for fatty acid synthesis via ACoA, NADPH and lipogenic enzymes such as ATP citrate lyase (ACLY), acyl-CoA synthetase/acid-CoA ligase (ACS/ACSL), ACoA carboxylase (ACC), fatty acid synthase (FASN) and stearoyl-CoA desaturase(Currie E et al., 2013). Glutamine is also a major source of carbons for the de novo synthesis of fatty acids. There are two pathways for this: glutaminolysis followed by the Krebs cycle pathway and reductive carboxylation, which follows a pathway opposite to the Krebs cycle, forming citrate from -KG. Under conditions of hypoxic stress, cancer cells use reductive carboxylation of glutamine to generate citrate to synthesise lipids and support cancer cell proliferation (Sun RC and Denko NC, 2014). In BRAFV600E-mutated melanoma, cells show an increase in SREBP-1 (Sterol Regulator Element Binding 1)-induced lipid synthesis compared to neonatal human epidermal melanocytes (Talebi A et al., 2018).

Covalent modifications by CoA via ACS. At this stage, GA-CoA can either enter the mitochondria via the CPT1 transporter and be oxidised, or be esterified with glycerol or sterol backbones to generate triglycerides (TGs) or sterol esters respectively by the glycerol-3-phosphate-acyltransferase (GPAT) enzymes, acyl-glycerolphosphate acyltransferase (AGPAT), phosphohydrolase (PAP), diacyl-glycerol acyltransferase (DGAT). TGs can then be incorporated into membranes, stored, used as signalling molecules or oxidised as an energy source by adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL) and monoacylglycerol lipase (MAGL). (Right) Model explaining how fatty acids can limit cancer cell proliferation by (1) blocking FA synthesis, (2) increasing FA degradation, (3) increasing FA storage in neutral triglycerides, and/or/ (4) decreasing FA release from storage (after Currie E et al., 2013).

V. Tumour angiogenesis

Angiogenesis corresponds to the formation of new vessels from a pre-existing network, thus differing from vasculogenesis which is an essentially developmental process during which a vessel is established de novo from cellular precursors (Suchting et al. 2007).

Angiogenesis is normally only observed under conditions or within strictly regulated physiological mechanisms such as tissue repair, the menstrual cycle, pregnancy or to meet specific oxygen and nutrient requirements. Alterations to these processes can lead to abnormalities in vascular homeostasis and in the tissue irrigated, often with consequences for major organ functions...

The formation of a vascular network dedicated to the supply of oxygen and nutrients is an integral part of the tumour signature (Hanahan and Weinberg 2011).

- Angiogenesis by budding (which is the most studied process) is particularly dependent on VEGF concentrations in the environment; the number of selected front cells and the number of filopodia appear to be abnormally high.

- Endothelial precursor cells, either circulating or resident in the bone marrow or vascular walls, could be recruited to the tumour and establish a de novo blood network by vasculogenesis.

- Vascular mimicry, the ability of tumour cells to directly integrate blood vessels and artificially and aberrantly increase vascular mass, has been demonstrated in highly aggressive tumours such as metastatic melanoma.

- Another mode of tumour vascularisation is directly related to the properties of tumour cells. It involves the transdifferentiation of undifferentiated cancer cells that have the character of stem cells, as has been observed in glioblastomas (Hanahan and Weinberg 2011).
- The bisection of a pre-existing blood vessel by intussusception is thought to contribute to the branching and chaotic architecture of tumour vessels. Whatever the extent of the vascularisation process deployed to irrigate the tumour mass, this is

generally inefficient and disorganised, but nevertheless contributes to the aggressiveness of the tumour and its possible dissemination.

- ***Structure and characteristics of tumour vessels***

Tumour vessels are characterised by high constitutive permeability, dilation, a chaotic network and aberrant blood flow (Figure 5). Within the same vessel, blood flow is not constant and can even change direction (Hanahan and Weinberg 2011).

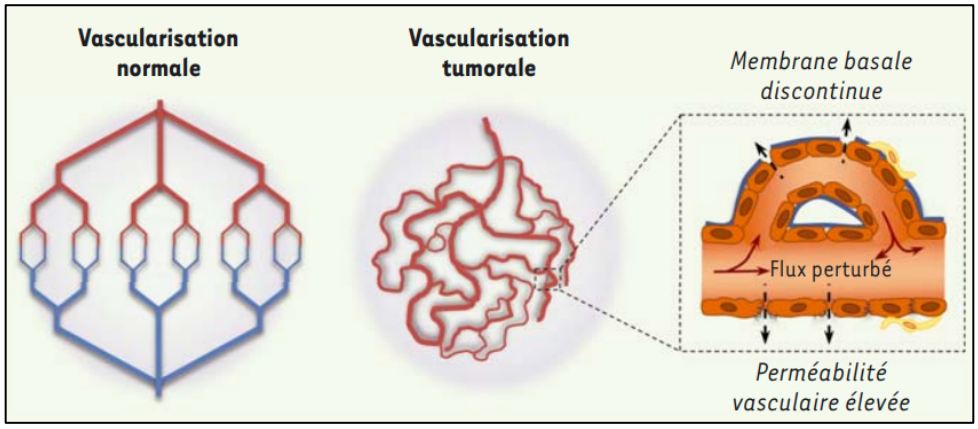


Figure 5 Les aberrations du réseau vasculaire tumoral. L'organisation générale d'un réseau vasculaire normal structuré et hiérarchisé, s'oppose à celle de la vascularisation aberrante et tortueuse observée dans les tumeurs. Les vaisseaux ont une perméabilité vasculaire anormale associée à une déstabilisation des protéines de jonctions comme la cadhérine endothéliale, une membrane basale discontinue, des péricytes peu nombreux et lâches, un flux sanguin inconstant et de multiples anastomoses vasculaires.

As a result of vascular permeability, interstitial pressure is high. This exacerbates oedema, fibrosis and inflammation. This in turn promotes angiogenesis and tumour dissemination (Azzi, Hebda and Gavard 2013). Tumour vessels form only a partial barrier to large molecules and plasma proteins.

In experiments involving the extravasation of different molecular weights, which enable vascular permeability to be measured, it can be shown that small molecules penetrate more deeply into tumour tissue but are rapidly eliminated. On the other hand, larger molecules (40-70 kDa) accumulate preferentially on the vessel surface, where they appear to persist (Dreher et al. 2006).

In addition to a lack of coverage by pericytes and a discontinuous basement membrane, the endothelial junctions in tumour vessels are also aberrant. The number of cells positive for the

smooth muscle cell marker D-SMA (smooth muscle actin) is reduced in xenograft models (Inai et al. 2004). Similarly, in ex vivo models of glioblastoma, the junctions formed by endothelial cadherin are disorganised (Bouvrée et al. 2012). Combined with a microenvironment rich in pro-angiogenic and inflammatory factors, and aberrations affecting the composition of tumour vessels, vascular permeability is abnormally high within tumours. These vascular leaks lead to significant interstitial pressure and the formation of vascular oedema, which makes drug addressing and delivery difficult and heterogeneous within the tumour (Dreher et al. 2006).

▪ ***The role of angiogenesis in tumour progression.***

Tumour angiogenesis quickly becomes paramount for tumour development. Defective tumour neo-vessels also participate in creating a unique metabolic and immune environment (Treps and Gavard 2015). Indeed, tumour angiogenesis helps to increase the influx of oxygen and nutrients, the supply of which by simple diffusion is no longer sufficient for tumour growth (oxygen can only diffuse passively over short distances, of the order of a hundred micrometres). The tumour environment is therefore enriched with numerous pro-angiogenic factors, for example in response to the activation of hypoxia pathways, the inflammatory microenvironment or metabolic or genetic aberrations in tumour cells (Carmeliet and Jain 2011a).

In addition to tumour growth, the conditions developed in the tumour environment exacerbate their aggressiveness. This is achieved by the selection within the tumour mass of the most pro-angiogenic cells, by metastatic dissemination, the acquisition of chemo- and radio-resistance, immune escape, etc. (Carmeliet and Jain 2011a). The recent discovery of stem-like tumour cells also suggests the existence of a tumour vascular niche (Calabrese et al. 2007). Cancer stem cells have been observed in the direct vicinity of endothelial cells. They could contribute to the maintenance of this tumour subpopulation with its unique properties (Galan-Moya et al. 2011). Against this backdrop, efforts in cancer research over the last few decades have led to the development of molecules aimed at blocking tumour angiogenesis in order to starve tumours and/or block metastatic exit pathways. In particular, bevacizumab, a VEGF-blocking antibody, has proved effective in metastatic colorectal and renal cancers in combination with chemotherapy

(Azzi, Hebda, and Gavard 2013). Its use is still debated in glioblastoma and breast cancer. Although it has a real anti-tumour action, bevacizumab could promote the acquisition of a more aggressive phenotype and metastatic dissemination, as has been shown in experimental models of glioblastoma and breast and pancreatic cancers (Carmeliet and Jain 2011b).
Mechanisms of resistance to anti-VEGF suggest that other pro-angiogenic molecules are involved in the tumour development process, or that tumour endothelial cells have become insensitive to VEGF for their growth.The role of inflammation and free radicals in tumour development

The process of carcinogenesis is traditionally considered to be a multi-stage process originating in the activation of oncogenes and the inactivation of tumour suppressor genes. However, the mechanisms underlying tumorigenesis cannot be reduced to molecular alterations alone, and need to be understood in their entirety, including the influence of the tumour environment.
The many advances in our understanding of tumours have shown that the process of tumorigenesis is the result of a long process consisting of a succession of stages in which a set of molecular events necessary for the transformation of a normal cell into a cancerous cell coexist (Basu 2018). In the case of the process of carcinogenesis, where the tumour originates from an epithelial cell, there are two major stages: tumour initiation and tumour progression.
Agents influencing tumour progression are not usually mutagenic and promote cell growth via various actions: pro-inflammatory effects, induction of mitotic signals, endocrine disruption effect.

▪ Role of the tumour microenvironment (tumour stroma)

Cancer should not be considered as a purely genetic and cellular pathology, but as a systemic disease whose evolution depends largely on the tumour's interactions with its immediate environment (Wang et al. 2017). The latter, referred to as the microenvironment or tumour stroma, includes both the constituents of the extracellular matrix and certain cell types that form, on the one hand, the mesenchymal compartment (fibroblasts, myofibroblasts, pericytes, endothelial and adipocyte cells) and, on the other,

the immune compartment (macrophages, dendritic cells, lymphocytes, plasma cells, polymorphs and mast cells) (Wang et al. 2017).

Given that the normal stroma negatively regulates the neoplastic process, the development of an invasive carcinoma involves the remodelling of its microenvironment, also known as stromal reaction or desmoplasia, to make it permissive and conducive to tumour development (DeClerck 2012). Furthermore, the nature of the tumour microenvironment as well as the intensity of the stromal reaction varies depending on the organ in which the tumour develops (Nissen, Karsdal, and Willumsen 2019). For example, tumours of the pancreas, breast and colon are characterised by the intensity of their stromal reaction (DeClerck 2012).

From a fundamental point of view, the desmoplastic reaction results, on the one hand, in an increase in the production of ECM (Extra-Cellular Matrix) rich in fibrillar collagen, and, on the other hand, in activation of each of the cellular components of the stroma leading to major destructuring of the tissue with the formation of an anarchic vascular system and recruitment of inflammatory cells, the whole forming a microenvironment favourable to tumour development (DeClerck 2012).

The activated stromal cells are mainly fibroblastic cells known as FAC (Fibroblasts Associated with Cancer) (Nissen, Karsdal, and Willumsen 2019).

These cells, via autocrine or paracrine signals, play a key role in the reorganisation of the ECM, tumour growth and dissemination, as well as in mechanisms of escape from the immune system, notably by secreting pro-angiogenic, pro-metastatic, anti-apoptotic or immunomodulatory factors (Nissen, Karsdal, and Willumsen 2019). Finally, in order to create the most favourable environment for its development, the cancerous tumour is capable of establishing reciprocal interactions not only with its surrounding cells, but also with distant tissues. For example, in some prostate cancers, the neuronal intratumoral network is partly formed by the migration of neuronal progenitors (Mauffrey et al. 2019).

- ***Inflammation***

While the acute inflammatory response, the body's main defence mechanism against aggression, promotes cell renewal and the restoration of tissue integrity, its persistence becomes harmful and in many cases pro-tumorigenic (Mauffrey et al. 2019).

In fact, inflammatory cells produce numerous pro-tumour molecules that play a major role in tumour initiation and progression, in particular free radicals resulting from oxidative stress secondary to the inflammatory reaction, pro-metastatic pro-angiogenic factors, immunomodulatory factors and inflammatory cytokines that promote tumour growth and survival (Elinav et al. 2013). In particular, activation of the transcription factors STAT3 and NF-κB via various inflammatory cytokines such as Il6 or TNFα play a major role in the tumorigenesis process (Elinav et al. 2013). In particular, chronic inflammation, whatever its cause, is considered to be a major risk factor for the occurrence of cancer. One of the best examples at present is that of the Helicobacter pylori bacterium, capable of inducing gastric cancer in part through the gastroduodenal production of pro-inflammatory cytokines such as IL-6 and TNFα and the induction of a chronic inflammatory response (Elinav et al. 2013).
Similarly obesity, characterised by chronic inflammation of adipose tissue, is another pathological condition well known to be associated not only with an increased incidence of cancers but also with aggressive tumours. Indeed, adipocyte cells, by secreting pro-inflammatory molecules, promote tumour development (Kolb, Sutterwala, and Zhang 2016).
Finally, some studies also suggest the involvement of the gut microbiota in the aetiology of cancers of the digestive tract, particularly colon cancers (Elinav et al. 2013). A major imbalance in the intestinal microbiota is thought to be responsible for the production of inflammatory toxins by certain bacteria, thereby promoting the development of colorectal cancer (Elinav et al. 2013).

Role of the tumour stroma

The stroma consists of the extracellular matrix (ECM), composed of proteoglycans, hyaluronic acid and fibrous proteins such as collagen, fibronectin and laminin, growth factors, chemokines, cytokines, antibodies and metabolites, and mesenchymal support cells (e.g. growth factors, chemokines, cytokines, antibodies and metabolites ; mesenchymal support cells (e.g. fibroblasts and adipocytes), cells of the vascular system and cells of the immune system. The stroma evolves as tumours develop (Lu, Weaver, and Werb 2012).

.1.Composition of stroma

Cancer cells produce factors that activate and recruit carcinoma-associated fibroblasts, which are a subtype of activated fibroblasts (myofibroblasts)(Kalluri and Zeisberg 2006). Carcinoma-associated fibroblasts resemble mesenchymal progenitors or embryonic

fibroblasts 8 and are capable of stimulating cancer cell growth and invasion as well as inflammation and angiogenesis (Kalluri and Zeisberg 2006). In some systems, they can also inhibit tumours (Kalluri and Zeisberg 2006). Carcinoma-associated fibroblasts activated by the tumour microenvironment are largely responsible for tumour-associated ECM changes, including increased ECM synthesis and remodelling of ECM proteins by proteinases, e.g. matrix metalloproteinases (MMPs) (Egeblad and Werb 2002). The modified ECM then influences tumour progression through architectural and signalling interactions (Nguyen-Ngoc et al. 2012).Several ECM proteins such as tenascin C and an alternatively spliced version of fibronectin expressed embryonically during organ development are re-expressed during tumour progression (Avraamides, Garmy-Susini, and Varner 2008). Type I fibrillar collagen also increases in tumours (Erler and Weaver 2009). Fragments of type I collagen or laminin 332 produced as a result of MMP cleavage may promote tumour growth by stimulating cell migration and survival (Avraamides, Garmy-Susini, and Varner 2008). The biophysical characteristics of tissues, such as rigidity, can affect cell function. Mammary epithelial cells cultured in compliant collagen matrices form polarisedacini, whereas in rigid matrices they lose their polarity and become proliferative and invasive (Paszek et al. 2005).inflammatory responses are associated with many cancers and can facilitate tumour progression (Coussens and Werb 2002). Adaptive and innate immune cells infiltrate tissues and play an essential role (DeNardo et al. 2011). While the innate immune compartment mainly promotes the tumour, the adaptive immune compartment (B and T cells) can suppress the tumour. The adaptive immune compartment (B and T cells) provides immune surveillance, controlling initiated cancer cells (DeNardo et al. 2011). Indeed, patients with a weakened adaptive immune system have an increased risk of developing cancer (de Visser, Eichten, and Coussens 2006). CD4 + T cells are key regulators of the immune system and differentiate into various helper T cell lineages: interferon γ-producing H 1 T cells that promote cell-mediated immunity and interleukin 4 (IL-4)-producing T helper 2 (H 2 T) cells that support humoral immune responses (Noy and Pollard 2014). H 1 T cells and H 2 T cells can enhance antitumour immunity by expanding the population of cytotoxic CD8 + T lymphocytes (CTCs). In contrast, regulatory T cells (Tregs) suppress antitumour immunity by inhibiting cytotoxic T cells. TH 17 cells secrete IL-17. While H 1 T cells are primarily anti-tumour, H 2 T cells promote tumours through their cytokines, which polarisetumour-

associated macrophages (TAMs) to promote cancer progression (DeNardo et al. 2009). CD4 + Tregs are immunosuppressive, directly suppressing the antitumour immunity of CD8 + cytotoxic T cells via secretion of IL-10 and transforming growth factor β. Depletion of Tregs enhances tumour growth (Noy and Pollard 2014). CD4 + T H 17 cells play a role in inflammation and tumour immunity. TH 17 cells develop from naive CD4 + T cells in the presence of transforming growth factor β, IL-6 and IL-1β. Whether TH 17 cells adopt a protumorigenic or antitumorigenic role depends on the stimuli encountered by the cells.

Innate cells of myeloid origin (e.g. macrophages, neutrophils and mast cells) are largely responsible for inflammatory responses (Fig. 6).

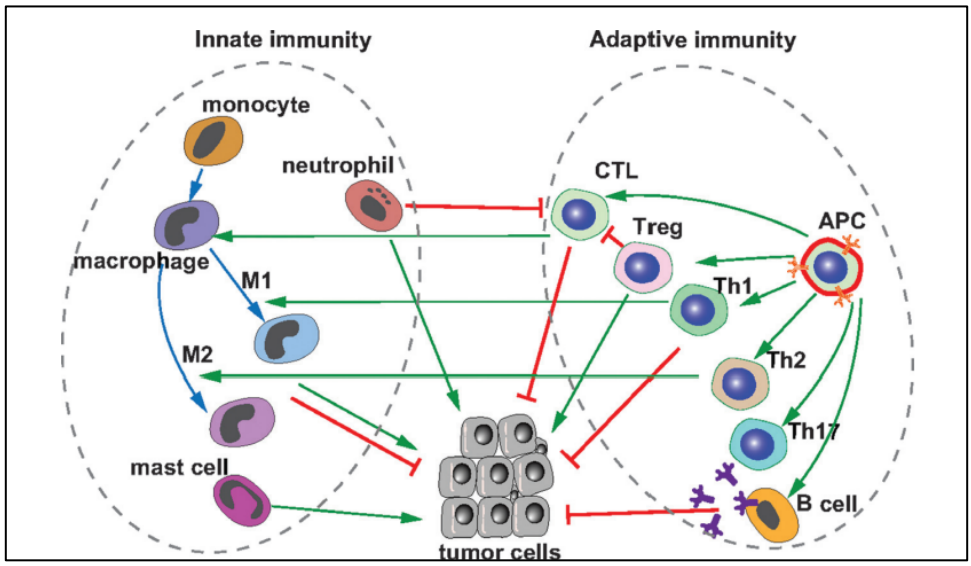


Figure 6 Multifaceted roles of innate and adaptive immunity in cancer development.While adaptive immunity, notably T and B lymphocytes, is essential for inhibiting cancer development, innate immunity, notably neutrophils, macrophages and mast cells, can promote or inhibit cancer development depending on local and systemic contexts. For example, macrophages can be polarized and activated by cytokines secreted by TH 1 cells, producing reactive oxygen and nitrogen intermediates and inflammatory cytokines. These pro-inflammatory M1 macrophages can inhibit tumorigenesis; in contrast, TAMs (or anti-inflammatory M2s) polarized by cytokines secreted by TH 2 cells are associated with a poor prognosis. Regulatory T lymphocytes can inhibit the function of cytotoxic T lymphocytes, thereby promoting tumorigenesis.

Monocytes are polarised into M1 macrophages by cytokines secreted by H 1 T cells such as interferon γ, tumour necrosis factor α and granulocyte-monocyte colony-stimulating factor; produce reactive oxygen and nitrogen intermediates and inflammatory cytokines; and are

antitumour (Mantovani et al. 2008). In contrast, monocytes exposed to cytokines secreted by TH 2 cells such as IL-4 and IL-13 polarise towards the M2 macrophage phenotype. However, this classification does not precisely define the differentiated state of macrophages exposed to complex environments in vivo. Tumour-associated macrophages predominantly resemble M2 macrophages. TAM accumulation is associated with a poor prognosis (Francesco Colotta et al. 2009).

- ***Autophagy***

Autophagy is a physiological cellular process for the degradation and elimination of misfolded proteins and damaged organelles that functions in developmental adaptation, cell death and tumour suppression (Mizushima 2007). One important mechanism of autophagy is an intracellular degradation pathway mediated by double-membrane vesicles called autophagosomes. These autophagosomes deliver degraded cytoplasmic components to the lysosome for recycling under conditions of stress. This autophagy mechanism is essential to protect cells from damaged proteins, to protect cellular organelles from toxins, to maintain cellular metabolism and energy homeostasis and to promote cell survival. Autophagy can be general (non-selective) or selective. General autophagy packages parts of the cytoplasm into autophagosomes and transfers them to lysosomes for degradation. In contrast, selective autophagy works by recognising specific targets, such as damaged cell organelles, protein aggregates and intracellular pathogens. Recently, it has been reported that defects in autophagy are associated with genomic damage, metabolic stress and tumorigenesis (Ameisen 2002). In addition, numerous studies have suggested that autophagy is linked to both cancer initiation and cancer treatment for several years (White 2012).

Indeed, some studies suggest that autophagy is a regulator of many oncogenes and tumour suppressor genes (Botti et al. 2006), while other studies have shown that autophagy is involved in both the promotion of tumorigenesis and the development and inhibition of cancer (Rosenfeldt and Ryan 2011) .

1.Overview of autophagy

Autophagy is an evolutionarily conserved intracellular recycling system and cellular self-degradation process that maintains metabolism and homeostasis. Autophagy responds to a range of cellular stresses, including nutrient deprivation, organelle damage and abnormal

protein accumulation (Russell, Yuan, and Guan 2014). This autophagic process can be associated with cell death and cell survival (Galluzzi et al. 2015). During nutrient deprivation, autophagy is enhanced to maintain a supply of important proteins and other nutrients to serve as an energy supply, thereby increasing cell survival (L. He et al. 2018). Recent studies have reported that hypoxia can regulate autophagy, inducing processes that mitigate oxidative stress caused by low oxygen levels (Fang, Tan, and Zhang 2015).

Under normal conditions, cells use basal levels of autophagy to help maintain biological function, homeostasis, quality control of cellular contents, and removal of old proteins and damaged organelles (L. Yu, Chen, and Tooze 2018). Moreover, autophagy in stem cells is linked to the maintenance of their unique properties, including differentiation and self-renewal (Pópulo, Lopes, and Soares 2012). In cancer cells, autophagy suppresses tumorigenesis by inhibiting cancer cell survival and inducing cell death, but it also facilitates tumorigenesis by promoting cancer cell proliferation and tumour growth.

The mechanism of the autophagic process is controlled by a series of proteins. The mammalian target of rapamycin (mTOR) is associated with cell proliferation, stress and cancer progression. mTOR consists of two complexes, mTORC1 and mTORC2, each of which has distinct functions and localisation (Pópulo, Lopes, and Soares 2012). activated mTORC1 plays a central role in the phosphorylation of autophagy-related protein (ATG) and leads to the inhibition of autophagy. When mTORC1 is inhibited under various stressful conditions, such as starvation and organelle damage, autophagy is enhanced. mTORC1 is regulated by AMP-activated protein kinase (AMPK), and inhibiting mTORC1 and increasing AMPK induces the autophagic process (Rosenfeldt and Ryan 2011). However, the role of mTORC1 in autophagy is unclear (Pópulo, Lopes, and Soares 2012).

When mTORC1 is inhibited, the Unc-51-like autophagy-activating kinase (ULK) complex is dephosphorylated and thus becomes activated (Torii et al. 2016). The activated ULK complex localises to the phagophore and activates class III PI3K (Itakura et al. 2008). Beclin-1 recruits numerous proteins involved in autophagosome maturation and elongation (Galluzzi et al. 2015).

Elongation of autophagosome formation is regulated by ATGs. ATG5 - ATG12/ATG16L complexes recruit microtubule-associated protein 1 light chain 3 (LC3) and are associated with phagophore expansion (Mochida et al. 2015). Subsequently, LC3 drives phagophore

elongation. Pro LC3 is converted to the active cytosolic isoform LC3 I by ATG4B. LC3 I is then converted to LC3 II by interacting with phosphatidylethanolamine (PE), ATG3 and ATG7. LC3 II is located in the inner and outer membrane of the autophagosome, allowing it to bind to degraded substrates (Kabeya et al. 2000). Mature autophagosomes can fuse with lysosomes to form autolysosomes, which selectively remove damaged proteins and organelles via autophagy (Thurston et al. 2016).

1I.The role of autophagy in cancer

Autophagy plays a major role in the degradation of damaged organelles and old proteins and in the maintenance of cellular homeostasis (Karsli-Uzunbas et al. 2014).

In cancer biology, autophagy plays a dual role in tumour promotion and suppression (Fig 7) and contributes to cancer cell development and proliferation. Certain anti-cancer drugs can regulate autophagy. As a result, autophagy-regulated chemotherapy may be involved in cancer cell survival or death (Rosenfeldt and Ryan 2011).

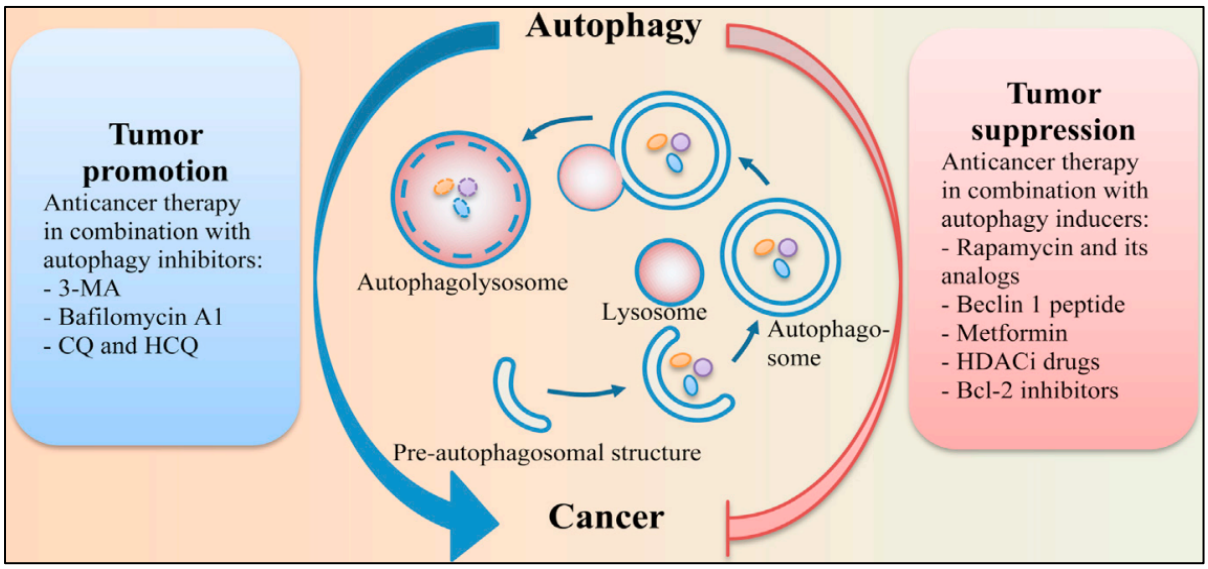


Figure 7 the dual role of autophagy in cancer. As a stress-sensitive cellular mechanism, autophagy can be either pro- or anti-cancerous. Consequently, autophagy inducers and inhibitors can be used to sensitize tumor cells to chemotherapeutic drugs (Rosenfeldt and Ryan 2011).

In addition, regulation of autophagy contributes to the expression of tumour suppressor proteins or oncogenes. Tumour suppressor factors are negatively regulated by mTOR and AMPK, leading to induction of autophagy and suppression of cancer initiation (Comel et al.

2014). In contrast, oncogenes can be activated by mTOR, class I PI3K and AKT, leading to suppression of autophagy and enhancement of cancer formation (Choi, Ryter, and Levine 2013).

Reduced and abnormal autophagy inhibits the degradation of damaged components or proteins in cells under oxidative stress, leading to cancer development. In addition, basal autophagy is considered to be a suppressor of cancer (Qu et al. 2003). Mutation of important autophagy proteins inhibits tumour development. It has been observed that BIF-1 proteins linked to BECN1 become abnormal or absent in various types of cancer, such as colorectal and gastric cancer (Coppola et al. 2008). UVRAG proteins are also linked to BECN1 and function as regulators of autophagy. UVRAG mutation reduces autophagy, leading to increased cancer cell proliferation in colorectal cancer cells (S. He et al. 2015). On the other hand, a high basal level of autophagy is observed in several RAS-activated cancer types, such as pancreatic cancers (Perera et al. 2015). Inhibition of increased autophagy in these cancers decreases cell proliferation and promotes tumour suppression (Yang et al. 2014).

- ***Telomeres, senescence and carcinogenesis***

Ageing is accompanied by an increasing number of senescent cells in the body. This process participates in the accumulation of functional alterations in tissues, leading to the onset of multiple age-related diseases (Hernandez-Segura, Nehme, and Demaria 2018).

The phenomenon of cellular senescence was described by Hayflick and Moorhead in 1961 as a cessation of normal cell division. These authors highlighted the limited mitotic capacity of normal human cells, thus introducing the concept of replicative senescence. Since these initial observations, the characterisation of this phenotype and the mechanisms by which it is induced by various cellular stresses have been largely clarified and fuelled by new knowledge. The majority of accelerated ageing syndromes are diseases mainly associated with a signalling or repair defect in DNA lesions or structural defects in the nuclear envelope. We shall see that a recurrent feature of a large number of senescence-inducing situations is the presence of DNA damage. In addition, the discovery of the pro-inflammatory secretion process of senescent cells has made it possible to establish a clear link between senescence and ageing at the level of the whole organism and to understand the common links between age-related diseases.

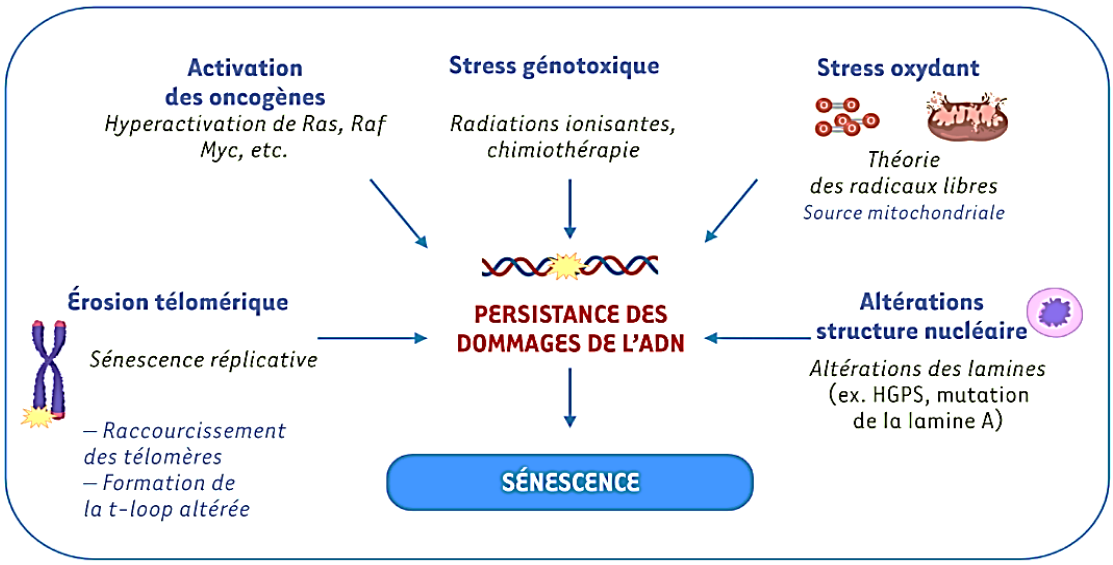


Figure 8 a common element between different senescence situations. Various situations leading to the induction of senescence are associated with the accumulation of DNA damage: telomere erosion (replicative senescence), oncogene hyperactivation (OIS), exposure (therapeutic or accidental) to genotoxic agents, oxidative stress and alterations in nuclear structure (**Fontanilla et al. 2020**).

1.Cellular senescence :

Cellular ageing can be defined as a signal transduction programme leading to the irreversible cessation of cell proliferation and accompanied by changes in the phenotype of the cell. Cell ageing is closely linked to DNA size. At its ends are DNA-protein structures called telomeres, which enable DNA repair enzymes not to confuse the end of the DNA with abnormal DNA breaks. The structure of telomeres protects the terminal end of chromosomes from damage in a way that is not well understood (Makarov, Hirose and Langmore 1997).

Cellular longevity appears to be linked to telomere size. In many organisms, DNA size is kept constant by telomerase, which recognises the ends of telomeres during DNA duplication (replication). This enzyme is present in germ cells, but is no longer expressed in most somatic cells (W.-Y. Yu et al. 2008). During cell multiplication, when the chromosome duplicates (replication), the replication systems cannot reach the end of the chromosome. This is because DNA polymerase is unidirectional and, in the absence of an RNA primer supplied by telomerase, the telomere shortens in size with each cell

multiplication. When the telomere reaches a critical size, the cell definitively stops multiplying and acquires morphological changes and an alteration in a number of biological functions known as cellular senescence. In fibroblast cell culture, this process leads to the secretion of extracellular matrix proteins, matrix degradation enzymes, inflammatory cytokines and growth factors (Dominiczak 2000). It is the suppressor genes P53 and RB that regulate the pathways leading to cell cycle arrest or apoptosis (Pommier and Kohn 2003). Genomic instability caused by telomerase shortening can lead to mutations in P53 or RB which can suppress the 'senescence' response to telomere shortening (Maudelonde 2004). If the RB pathway is altered but P53 functions normally, the cell will undergo apoptosis. On the other hand, if P53 is also affected, genomic instability is no longer under control and the risk of carcinogenesis becomes high if the cell does not die from these accumulated genetic anomalies. Hyperactivity of P53 in rodents leads to accelerated ageing with a reduction in the frequency of cancers, underlining the important role of P53 in the ageing process and the link between the mechanisms of cellular senescence and carcinogenesis (Campisi 2004). Othermechanismscan lead to senescence, such as oxidative stress (Park et al. 2018).Mécanismes communs au vieillissementet au cancer

II. Telomere shortening

In human cell culture there is a progressive shortening of telomeres (López-Alcorocho et al. 2019). When tissues donated for transplants by elderly donors are analysed, a high frequency of shortened telomeres and senescent cellular characteristics are observed (Maudelonde 2004). In contrast, telomere shortening is not observed in murine cell cultures or in vivo in rodents (Shay and Bacchetti 1997). However, if telomerase is inactivated in mice, telomeres shorten within a few generations. When their size approaches that of human cells, mice develop c a n c e r s very similar to those of humans (Artandi et al. 2000).

Certain human pathologies, such as dyskeratosiscongenita, which presents a deficiency in one of the components of telomerase, are partially deficient in telomerase and have an increased incidence of cancer (Dez et al. 2003). On the other hand, people suffering from this pathology have biological abnormalities normally associated with age (immune ageing, greying and sparse hair, delayed wound healing (Rudolph et al. 1999).

- ***Cell cycle and cancer***

  Cancer encompasses many different diseases caused by a common mechanism: uncontrolled cell growth. Despite the levels of redundancy and overlap in cell cycle control measures, errors can occur. One of the critical processes monitored by the cell cycle checkpoint system is the proper replication of DNA during S phase. Even if all the cell cycle controls are working correctly, a small percentage of replication errors (or mutations) will be passed on to the daughter cells. If changes to the DNA nucleotide sequence occur in the coding part of a gene and are not corrected, a genetic mutation can occur. All cancers begin when a genetic mutation produces an abnormal protein that plays an important role in cell reproduction. The cellular change that results from the abnormal protein may be minor: perhaps late binding between Cdk and cyclin or detachment of an Rb protein from its target DNA while it is still in a phosphorylated state. However, even small errors can make it easier for subsequent errors to occur. Over time, small uncorrected errors are passed from a mother cell to daughter cells and amplified, as each generation produces more and more defective proteins from damaged DNA that has not been repaired. After a while, the cell cycle accelerates because the control and repair mechanisms become less effective. The uncontrolled growth of cells carrying the genetic mutation outstrips that of normal cells in the same area, and a tumour may result.

- ***Proto-oncogenes***

Genes that code for proteins involved in the positive regulation of the cell cycle are called proto-oncogenes. Proto-oncogenes are normal genes that, after undergoing a particular mutation, become oncogenes - genes that cause a normal cell to turn into a cancerous one. Take a look at what can happen in the cell cycle of a cell that has recently contained an oncogene. In some cases, the change in DNA sequence will result in the production of a protein that functions less well (or not at all). The result is harmful to the cell and will probably prevent it from completing its cell cycle; however, the organism will not suffer any harm because the mutation will not go any further. If a cell cannot reproduce, the mutation does not spread and the damage is minimal. Sometimes, a genetic mutation can still cause a change that increases the activity of a positive regulator. For example, a mutation that allows a Cdk to be activated without being paired with a cyclin could allow the cell to pass through a

checkpoint before all the necessary conditions have been met. If the resulting daughter cells are too damaged to undergo further cell divisions, the mutation would not be propagated and the organism would not suffer any harm. However, if the atypical daughter cells are allowed to undergo further cell divisions, subsequent generations of cells may accumulate more mutations, and some of these mutations may affect other genes involved in cell cycle regulation. The Cdk gene in the example above is one of many genes that are considered to be proto-oncogenes. In addition to cell cycle regulatory proteins, any protein that influences the cycle can be modified in such a way that it can bypass the cell cycle checkpoints. An oncogene is a gene that, when altered, leads to accelerated cell cycle progression

• ***.Tumour suppressor genes***

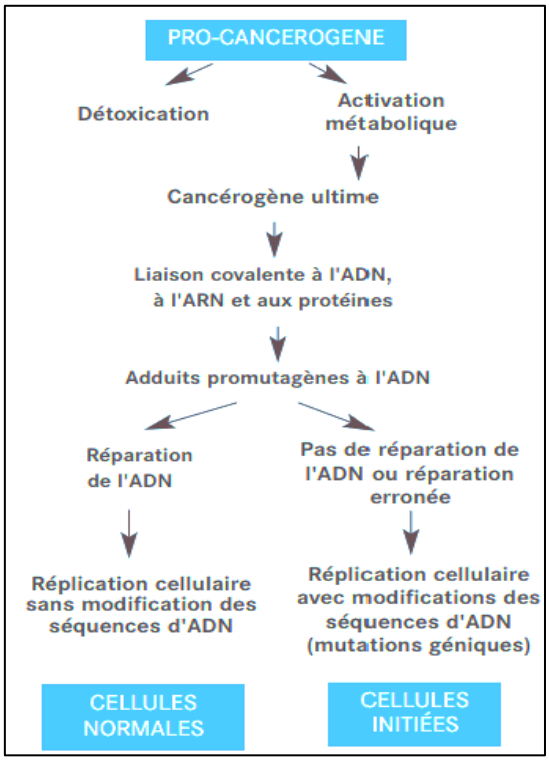
Like proto-oncogenes, many proteins involved in the negative regulation of the cell cycle have been discovered in cells that have become cancerous. Tumour suppressor genes are segments of DNA that code for negative regulatory proteins, the type of regulators that, when activated, can prevent a cell from undergoing uncontrolled division. The collective function of the best-known tumour suppressor gene proteins, Rb, p53 and p21, is to act as a barrier to cell cycle progression until certain events have been completed. A cell carrying a mutated form of negative regulator may not be able to interrupt the cell cycle if something goes wrong. In more than 50 per cent of human tumour cells, the p53 genes have been found to be mutated. This finding is not surprising given the multiple roles of the p53 protein at the G1 checkpoint. A cell with abnormal p53 may not detect errors in the genomic DNA. Even if a partially functional p53 is able to detect mutations, it may no longer be able to send a signal to the enzymes required for DNA repair. In any case, the damaged DNA will remain unrepaired. At this point, a functional p53 would judge that the cell is not salvageable and would trigger the programmed death of the cell (apoptosis). However, the damaged version of p53 found in cancer cells cannot trigger apoptosis.

The loss of p53 function has other repercussions on the cell cycle. Having undergone a mutation, p53 may be unable to trigger the production of p21. In the absence of adequate levels of p21, Cdk activation is not effectively blocked. In essence, in the absence of a fully functional p53, the G1 checkpoint is severely compromised and the cell can go directly from G1 to S, whether or not internal and external conditions are met. At the end of this shortened cell cycle, two daughter cells are born, having inherited the mutated p53 gene. Given the poor

conditions under which the mother cell reproduced, it is likely that the daughter cells will have acquired other mutations in addition to the defective tumour suppressor gene. Cells such as these daughter cells rapidly accumulate both oncogenes and malfunctioning tumour suppressor genes. Again, the result is tumour growth.Mecanismes de réparation des cassures a l ADN et cancer

Experimental studies on rodents and cells in culture have led to the classification of chemical carcinogens into two broad categories: genotoxic and non-genotoxic..

Genotoxic carcinogens modify the structure of DNA, mainly by covalent bonds at nucleophilic sites. These lesions, i.e. the chemical entity of the carcinogen bound to the DNA, are called DNA 'adducts'. Replication of DNA containing unrepaired adducts can either result in sequence changes (mutations) in the newly synthesised daughter DNA molecules, or in DNA rearrangements manifested aschromosomal aberrations.



This irreversible and critical genetic event can lead to the fixation of the original structural modification in the DNA, resulting in the presence of a permanent and transmissible genetic lesion, or the loss of genetic information via chromosomal alterations. This heritable modification, sometimes referred to as the 'initiation' stage of the tumorigenic process (fig.9), can disrupt growth control in the affected cell..

Figure 9 Critical steps in the initiation process

Activation of carcinogens was the first indication of an association between certain cancers and exposure to chemicals, based on observations made by clinicians in the eighteenth and nineteenth centuries. The field of experimental chemical carcinogenesis began in 1915 with the experiments of Yamagiwa and Ichikawa, who demonstrated that the application of tar to

rabbit ears induced skin tumours. In the 1940s, experiments on mouse skin established the stepwise evolution of cancer and made it possible to define two classes of agents, initiators and promoters (Miller and Miller 1979). Most carcinogens undergo metabolism, which leads to their elimination, but during which reactive intermediates are generated. This metabolic activation results in the modification of cellular macromolecules (nucleic acids and proteins) (Trosko and Chang 1984). As a result, mutagenicity tests using bacteria and mammalian cells in culture have been developed and are widely used to identify potential carcinogens. However, it is not possible to demonstrate that all chemicals known to cause cancer bind to DNA and therefore classify them as 'genotoxic'. The activation of chemical carcinogens in mammalian tissue is mainly the result of oxidation by microsomal mono-oxygenases (cytochromes P450, phase I enzymes). Cytochromes P450 are located in the endoplasmic reticulum (inner membranes of the cell) and form a protein superfamily; around 50 are currently known in humans. The oxidation products are substrates for other families of enzymes (transferases, phase II enzymes) which bind the carcinogenic residues to a glutathione, acetyl, glucuronide or sulphate group. The resulting conjugates are hydrophilic and can therefore be easily excreted.

Carcinogenic electrophilic metabolites are intermediate products of these metabolic reactions. The metabolic pathways are well characterised for the main classes of chemical carcinogens, including polycyclic aromatic hydrocarbons, aromatic amines, N-nitrosamines, aflatoxins and vinyl halides, which give rise to electrophilic species by phase I activation (Guengerich 2000). Other metabolic pathways are also known. For example, dihaloalkanes are activated into carcinogenic metabolites by glutathione S-transferases. Our understanding of carcinogen-DNA interactions (fig. 10) is largely due to the development of sensitive and specific methods for determining DNA adducts.

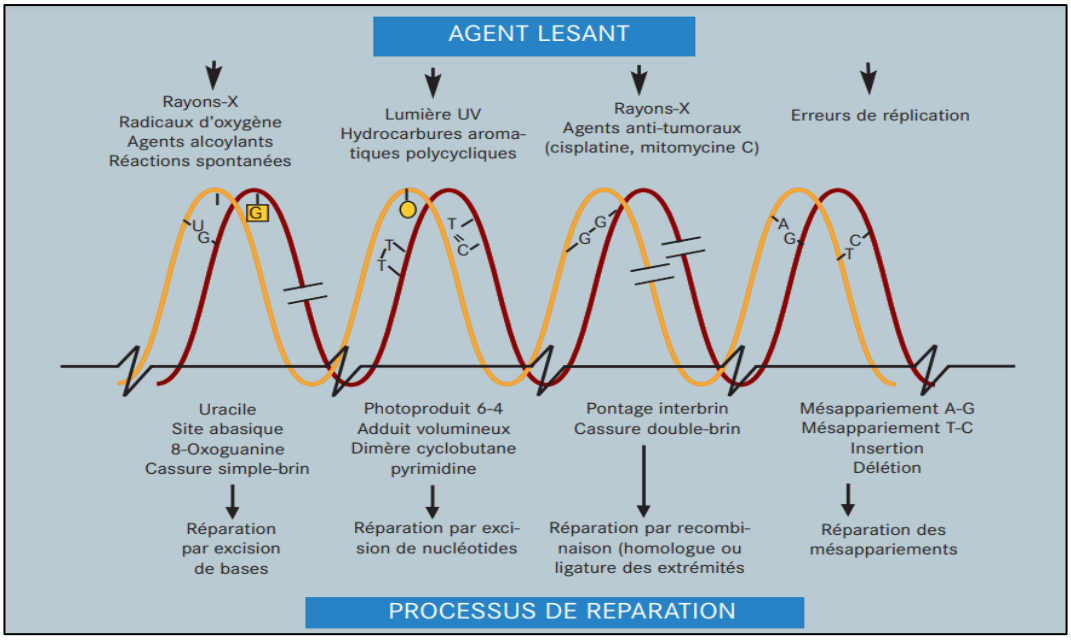


Figure 10 Agents that frequently damage DNA, examples of DNA lesions induced by these agents and the main DNA repair mechanisms responsible for suppressing these lesions.

The most frequently used methods are immunoassays using adduct-specific antisera or antibodies, p32 post-labelling, fluorescence spectroscopy, electrochemical detection and mass spectrometry. The measurement of carcinogen-DNA adducts in rodents has revealed correlations between the concentration of carcinogens in the environment, the levels of DNA adducts in tissues where tumours exposure, and therefore of carcinogenic risk to humans (T. P et al., n.d.).

However, the analysis of DNA adducts remains difficult in human cells and tissues due to the very low levels of adducts present in DNA (most often one adduct per 107 to 108 parent nucleotides). The enzymatic activities involved in the metabolism of carcinogens vary greatly from one individual to another due to induction and inhibition processes or genetic polymorphisms that can modify them. These variations can affect the formation of carcinogen-DNA adducts, in conjunction with other genetic determinants that regulate DNA repair or cell cycle control, for example, and can thus have an impact on the consequences of exposure to DNA-damaging agents and influence the risk of cancer in different individuals (V. P et al., n.d.). Many studies have attempted to correlate genetic polymorphisms, adduct levels and cancer risk in human populations. These studies have so far provided some correlations for risk prediction at the population level. However,

due to the large number of enzymes and polymorphisms involved, large-scale studies and high-throughput analyses (based on DNA microarrays, for example) will be needed to fully elucidate the complex nature of these gene-environment interactions. The most frequently used methods are immunoassays using adduct-specific antisera or antibodies, p32 post-labelling, fluorescence spectroscopy, electrochemical detection and mass spectrometry. The measurement of carcinogen-DNA adducts in rodents has revealed correlations between the concentration of carcinogens in the environment, the levels of DNA adducts in tissues where tumours can develop, and the incidence of cancer. It was therefore accepted that DNA adducts could be used as indicators of actual biological exposure, and therefore of carcinogenic risk to humans (T. P et al., n.d.).

However, the analysis of DNA adducts remains difficult in human cells and tissues due to the very low levels of adducts present in DNA (most often one adduct per 107 to 108 parent nucleotides). The enzymatic activities involved in the metabolism of carcinogens vary greatly from one individual to another due to induction and inhibition processes or genetic polymorphisms that can modify them. These variations can affect the formation of carcinogen-DNA adducts, in conjunction with other genetic determinants that regulate DNA repair or cell cycle control, for example, and can thus have an impact on the consequences of exposure to DNA-damaging agents and influence the risk of cancer in different individuals (V. P et al., n.d.). Many studies have attempted to correlate genetic polymorphisms, adduct levels and cancer risk in human populations. These studies have so far provided some correlations for risk prediction at the population level. However, due to the large number of enzymes and polymorphisms involved, large-scale studies and high-throughput analyses (based on DNA microarrays, for example) will be needed to fully elucidate the complex nature of these gene-environment interactions.

- ***Spectra of mutations***

As already indicated, it is possible to use DNA and protein adducts as early markers of exposure to carcinogens. However, since adducts persist for only a short time (a few hours to a few days for DNA adducts, a few weeks to a few months for albumin or haemoglobin adducts), their usefulness as exposure markers is limited. Mutations in specific genes can be used as longer-term 'biomarkers' of early biological effects or disease (Iarc and McGregor 2000). Indeed, mutation spectra are probably the only biological marker that can be

characteristic of past exposure to a carcinogenic agent or mixture. The study of these mutations will increasingly facilitate the identification of these etiological agents in cancer risk prediction and prevention studies. Mutation spectra can be analysed either in normal tissues (including blood cells) or in tumour tissues. Analysis of mutations in normal tissues remains difficult because the mutant cell or mutant DNA must be identified in the midst of a very large number of non-mutant cells or non-mutant DNA, and a selection or enrichment step is then necessary. On the other hand, mutations in tumour cells often promote growth and are amplified by clonal expansion of the tumour cell population. Some genes are suitable markers ('reporters') for the induction of a mutation in laboratory animals and humans. For example, the hypoxanthine-guanine phosphoribosyl-transferase gene (HPRT gene), when inactivated by mutation, renders cells resistant to growth inhibition by 6-thioguanine. It is therefore possible to isolate these mutant cells by culturing them in the presence of this agent. Studies in humans have associated the increased frequency of mutations in the HPRT gene (measured in circulating lymphocytes) with exposure to environmental genotoxic agents. However, unlike the observations made in rodents, in which mutation profiles often reflect the relatively extreme DNA lesions that induced them, the mutation spectra characteristic of the HPRT gene (i.e. the types and positions of base modifications within the HPRT gene DNA sequence) are more difficult to observe in humans. The identification of oncogenes and tumour suppressor genes has made it possible to characterise the genetic mutations most directly associated with carcinogenesis. The RAS family of oncogenes was one of the first to be identified as carrying mutations in a wide variety of human cancers. The p53 gene is the most frequently altered tumour suppressor gene in human cancer, being mutated in over 50% of virtually all tumour types. A large database of all p53 mutations has been created. Spectra of mutations have been identified that highlight the direct action of environmental carcinogens in the development of certain cancers (in this case it is possible to establish a causal relationship between cancer and past exposure to a specific carcinogen). These mutations, which could in principle be used to identify exposure to particular agents, have been called 'characteristic'

mutations, which result from the formation of specific DNA adducts. For example, mutations in p53, characteristic of the known or suspected aetiological agent, occur in lung cancer

(possibly attributed to benzo[a]pyrene in tobacco smoke) and hepatocellular carcinoma (due to aflatoxin B1 in contaminated food).

However, it is not always practical to obtain DNA from healthy tissue to analyse potentially tumorigenic mutations, as invasive sampling procedures are required. Fortunately, the protein products of the mutated genes, or even the mutated DNA itself, can be detected and measured in body fluids or secretions such as blood plasma, which have been in contact with the malignant tissue. Presumed characteristic mutations have also been identified in 'normal' tissues (non-pathological but probably containing initiated cells) of exposed individuals. For example, the p53 mutation associated with exposure to aflatoxin B1 was found in the liver tissue and plasma DNA of healthy (non-cancerous) subjects who had consumed aflatoxin-contaminated food. As a result, mutations in cancer genes could be used, in certain cases, as early indicators of risk before the disease is diagnosed.

can develop, and the incidence of cancer. It was therefore accepted that DNA adducts could be used as indicators of actual biological

- ***DNA repair***

The 3 x 109 nucleotides of DNA inside each human cell are constantly exposed to a range of damaging agents of both environmental (exogenous) origin, such as sunlight or tobacco smoke, and endogenous origin, such as water or oxygen (Crean, Mills, and Savage 2017).

This scenario requires constant monitoring to ensure that damaged nucleotides are removed and replaced, before their presence in a DNA strand leads to the appearance of mutations (Lindahl 2000). Restoration of the normal DNA structure is achieved in human cells by one of the few DNA repair enzymes that excise damaged or mismatched bases and replace them with a normal nucleotide sequence. This is known as 'excision repair'. Two main repair pathways function in this way: 'base excision repair', which operates mainly on modifications caused by endogenous agents, and 'nucleotide excision repair', which suppresses lesions induced by environmental mutagens. Ultraviolet light is probably the most common exogenous mutagen to which human cells are exposed, and the importance of the nucleotide excision repair pathway in protecting against UV-induced carcinogenesis is clearly demonstrated in the hereditary disease 'xerodermapigmentosum'. Individuals affected by this disease lack one of the enzymes involved in nucleotide excision repair, and are 1000 times

more likely to develop skin cancer after exposure to the sun than normal individuals. The genes in question have been named XPA, XPB, etc. (de Boer and Hoeijmakers 2000).

One of the greatest achievements of recent decades has been the isolation and characterisation of the genes and their protein products involved in base excision repair and nucleotide excision repair. It became clear that certain proteins identified in this way were not exclusively involved in DNA repair, but also played an integral role in other cellular processes such as DNA replication and recombination.

Excision repair

The first step in both base excision repair and nucleotide excision repair is the recognition of a modification in the DNA by enzymes which detect either specific forms of lesion, or a distortion in the DNA helix. Recognition of a lesion is followed by an excision step, during which the DNA containing the modified nucleotide is removed. DNA strand closure by synthesis and ligation of the free ends completes the DNA repair process. Nucleotide excision repair can take place in non-transcribed (non-protein-coding) regions of the DNA (**fig. 11, steps I to V**).

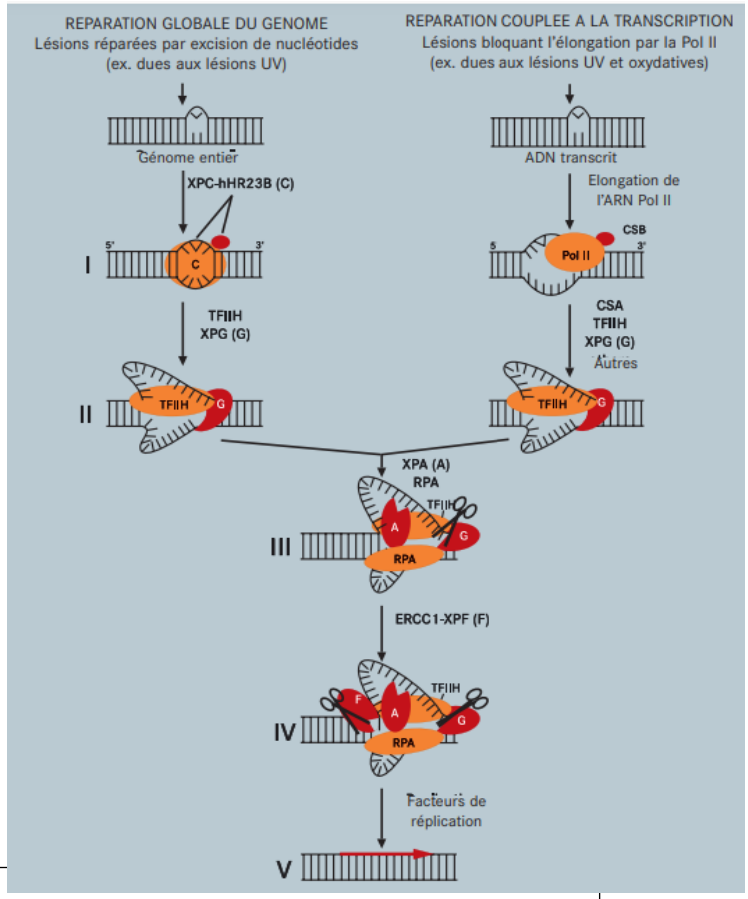


Figure 11 Nucleotide excision repair (NER). Two NER pathways predominate in removing DNA damaged by UV light and carcinogens. In genome-wide NER, the lesion is recognized by the XPC and hHR23B proteins, while in NER coupled to the transcription of protein-coding genes, the lesion is recognized when it blocks RNA polymerase II. After recognition, the two pathways are similar. The XPB and XPD helicases of the multi-subunit transcription factor TFIIH unwind DNA around the lesion (II). The single-strand binding protein RPA stabilizes the intermediate structure (III). XPG and ERCC1-XPF cleave the edges of the damaged strand, generating a 24-32 base oligonucleotide containing the lesion (IV). The DNA replication mechanism then ligates the free ends (V).

A DNA distortion is recognized, probably by the XPChHR23B (I) protein. An open bubble structure is then formed around the lesion in a reaction that involves the ATP-dependent helicase activities of XPB and XPD (two of the TFIIH subunits) and also recruits XPA and RPA (II-III). XPG and ERCC1-XPF nucleases excise and release an oligonucleotide of 24 to 32 residues (IV), DNA strand closure is achieved by ε- and δ-PCNA-dependent polymerases (POL) and free ends are ligated by a DNA ligase, presumed to be LIG1 (V). Repair by excision of nucleotides in regions that are transcribed (and therefore code for proteins) requires the action of TFIIH (Benhamou and Sarasin 2000).

Base excision repair (**fig. 12, stages I to VI or stages III to IX**) involves the removal of a single base by cleavage of the sugar-base bond by a DNA glycosylase.

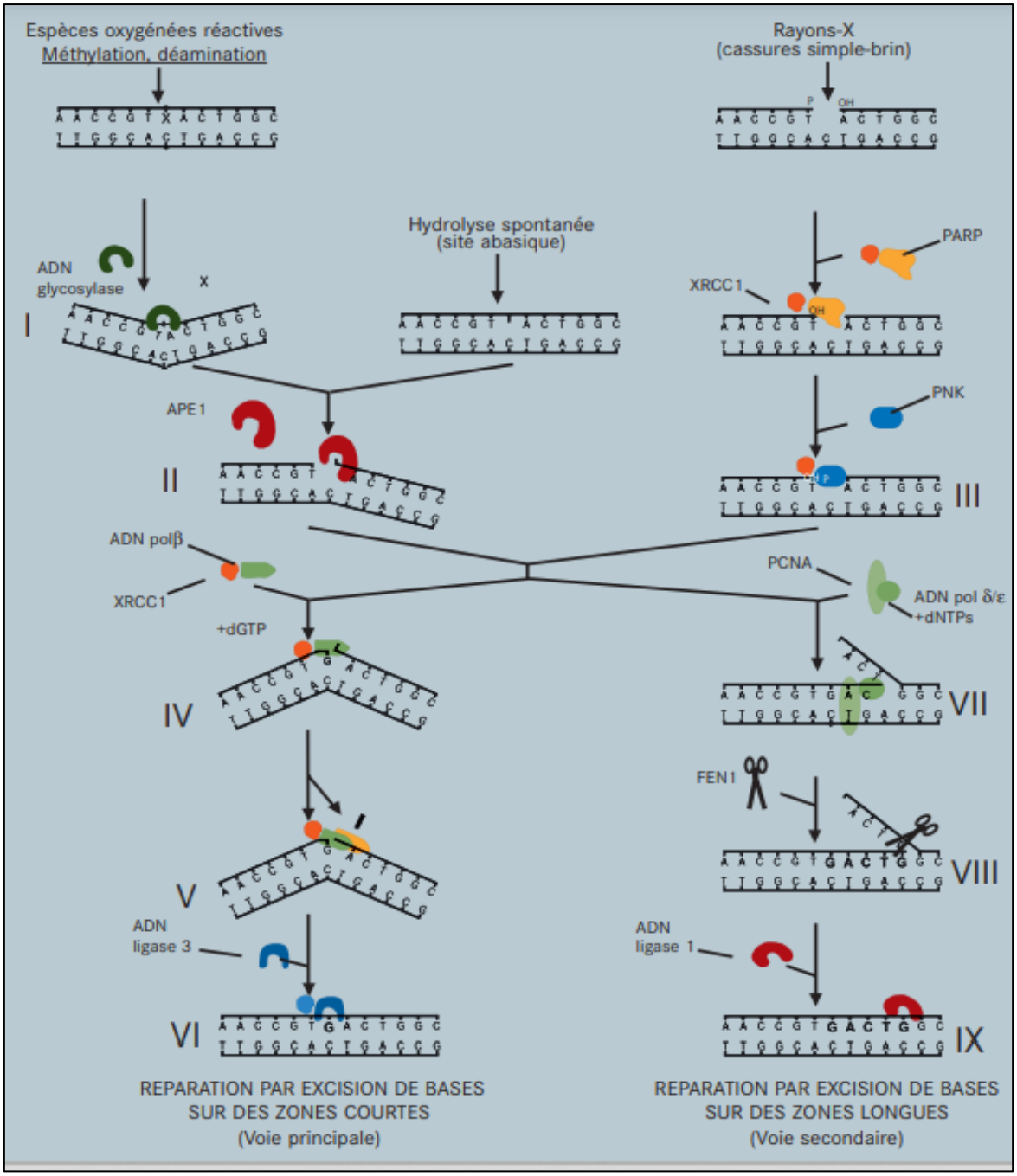


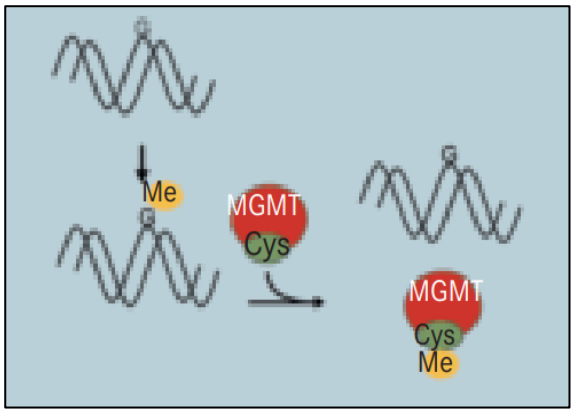
Figure 12 Stages of base excision repair. A large number of glycosylases are involved, each dealing with a relatively narrow spectrum of lesions. The glycosylase compresses the DNA backbone to propel the suspect base out of the DNA helix. The damaged base is cleaved inside the glycosylase, producing an 'abasic' site (I). The APE 1 endonuclease cleaves the DNA strand at the abasic site (II). In the repair of single-strand breaks, poly(ADP-ribose)polymerase (PARP) and polynucleotide kinase (PNK) may be involved. In the short-zone repair pathway, DNA polymerase β fills the gap left by the single nucleotide, and the ends of the single-strand break are ligated by DNA ligase 3. The long-zone repair pathway requires cell proliferative nuclear antigen (PCNA), and polymerases β, ε and δ fill the gap left by the 2-10 nucleotides. Flap endonuclease (FEN-1) removes the DNA fragment containing the lesion, and the strand ends are ligated by DNA ligase 3.

(for example, hNth1 or uracil DNAglycosylase) and incision by an apurinic/apyrimidinic nuclease (human AP1) (Cadet et al. 2000). DNA strand closure may be achieved by

replacement of a single base or by re-synthesis of several bases in the damaged strand, depending on the pathway used. More complex and less common forms of DNA damage, such as double-strand DNA breaks, clustered sites of base damage and non-coding lesions that block the normal replication process, are dealt with by other mechanisms. Inherited human pathologies in which patients show extreme sensitivity to ionizing radiation and an altered response to strand breaks, such as ataxia telangiectasia and Nijmegen syndrome, provide useful models for studying the repair enzymes involved in these processes. Indeed, if the elucidation of base excision repair and nucleotide excision repair was the most important advance of the late 1990s, then understanding strand break repair will probably be the most decisive advance of the decade to come. This will have far-reaching consequences.

Certain cancers are often treated with radiotherapy, but a small percentage of patients show considerable sensitivity to this treatment, making it necessary to limit the treatment protocol to avoid adverse reactions. A better understanding of the reasons for this radiosensitivity, and in particular the characterization of the enzymes involved in the repair of DNA lesions produced by ionizing radiation, may lead to better personalization of radiotherapy doses for patients.

Other repair methods :



Human cells, like eukaryotic and prokaryotic cells, can also perform a very specific form of lesion repair, converting the methylated adduct O6-methylguanine into a normal DNA base (**fig. 13**). O6-methylguanine is a misreading lesion: neither RNA nor DNA polymerase 'read' it correctly when transcribing or replicating a DNA template containing it.

Figure 13 Repair of O6-methylguanine by

Since this modified base can pair with both cytosine (its normal partner) and thymine (a mismatch partner), its presence in DNA can give rise to transitional mutations by mismatching the bases involved. A specific protein, O6-alkylguanine-DNA-alkyltransferase, catalyzes the transfer of guanine's methyl group to a cysteine amino acid residue located at the protein's active site.

This faithful process restores the DNA to its original state, but causes inactivation of the repair proteins. As a result, repair can be saturated when cells are exposed to high doses of alkylating agents, and protein transferase synthesis is required for repair to continue. Base mismatches in DNA arising from errors during DNA replication, for example the pairing of guanine to thymine rather than cytosine, are repaired by several pathways involving either specific glycosylases that remove the mismatched bases, or large-area mismatch repair using homologs of the bacterial MUTS and MUTL genes (**fig. 14**).

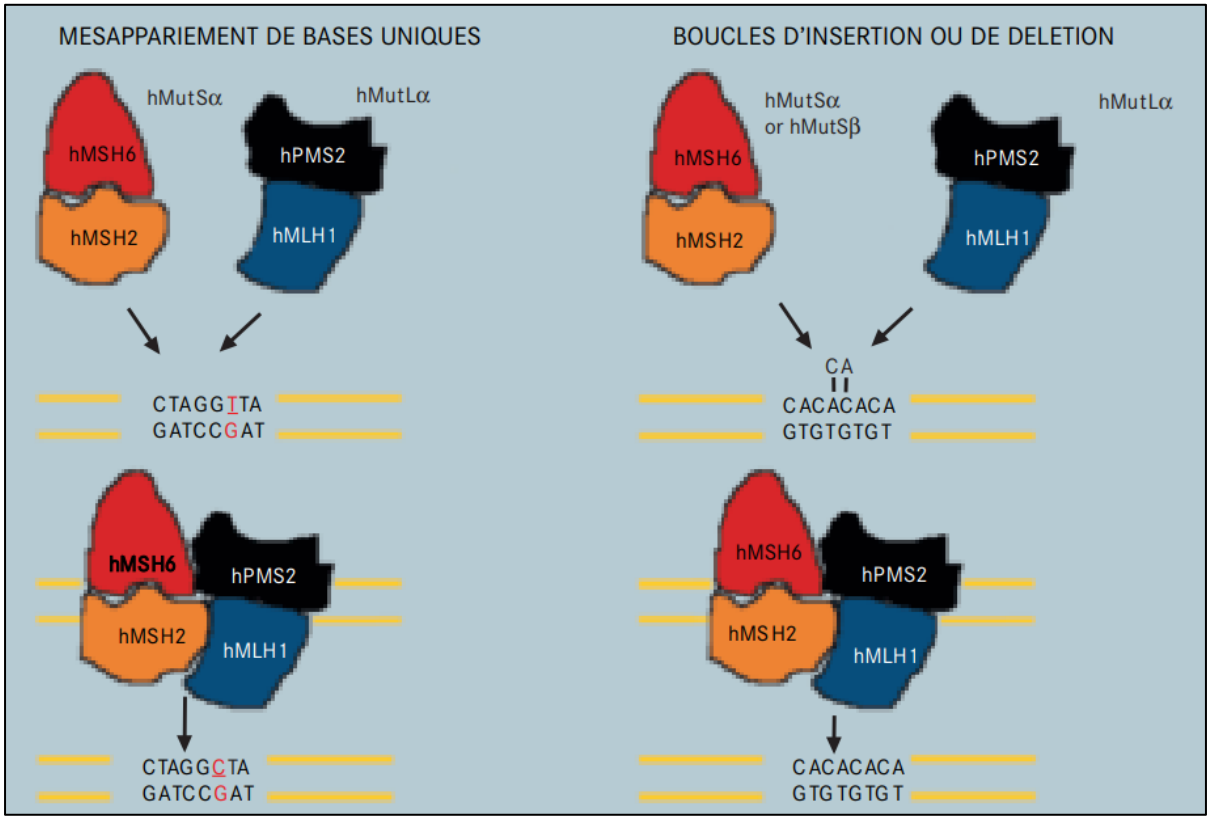


Figure 14 Mismatch repair pathways After DNA synthesis, base-pairing errors that have escaped the correction function of DNA polymerase are recognized by mismatch repair proteins.

Deletion or insertion loops at microsatellite sequences can be recognized by hMutSα (a heterodimer of hMSH2 and hMSH6) or by hMutSβ (a heterodimer of hMSH2 and hMSH3). Subsequent recruitment of hMutLα (a heterodimer of hMLH1 and hMLH2) to the altered DNA targets the area to be repaired that requires excision, resynthesis, and ligation. Recognition of single-nucleotide mismatch events requires the function of hMutSα. Importantly, these repair processes are able to differentiate between a correct and an incorrect base in the mismatch. Since both bases are normal constituents of DNA, this differentiation cannot be achieved by an enzyme that searches the DNA for a lesion or structure that is not a normal DNA constituent. Defects in at least four of the genes whose products are involved in mismatch repair, namely hMSH2, hMLH1, hPMS1 and hPMS2, have been associated with hereditary non-polyposis colorectal cancer. This is one of the most common genetic pathologies, affecting up to 1 in 200 individuals and accounting for 4-13% of all colorectal cancers.

Affected individuals also develop tumors of the endometrium, ovaries and other organs. The DNA of hereditary non-polyposis colorectal cancer tumors is characterized by instabilities in the mono-, di- and trinucleotide repeats that are common in the human genome.
This instability can also be observed in some sporadic colorectal tumor cells. It is the direct consequence of alterations in proteins involved in mismatch repair (V. P et al., n.d.).
Generally speaking, genomic instability is considered to be an indicator of malignant cell growth, and a fundamental determinant of its nature.

Chapter IV

Pathophysiology of hematological malignancies

Hereditary predisposition to carcinogenesis

Genetic cancer susceptibility syndromes, including those predisposing to leukemia and lymphoma, have been increasingly identified in recent years. Through clinical studies of affected individuals and families, and functional investigations of associated germline mutations, knowledge is emerging about the phenotypes of these syndromes, the biological mechanisms of tumor formation, and the effects of mutations on treatment response and tolerance. The information obtained is guiding the development of tailored approaches to oncology care, including modifications to cancer treatment and the incorporation of surveillance and risk-reduction measures, with the overall aim of reducing the morbidity and mortality associated with hereditary neoplasms. Here, we provide an overview of genetic cancer susceptibility syndromes, focusing on aspects relevant to hematopoietic malignancies.

Cancer is originally a genetic disease resulting from the accumulation of mutations that deregulate cell differentiation, proliferation and/or survival. In the majority of human cancers, these mutations occur in a single postzygotic cell. Nevertheless, the existence of cancer-prone parents suggests that some human cancers have a hereditary basis. This possibility was first recognized over 100 years ago, when in 1866, Paul Broca reported a large kinship including several members with breast cancer (Rahman 2014). Subsequently, other families characterized by distinctive patterns of cancer onset, many of which appear early or involve multiple primary tumors in the same individual, were described by Aldred Warthin and Henry Lynch (hereditary colon cancer without polyposis) and Frederick P. Li and Joseph Fraumeni. (Li-Fraumeni syndrome [LFS]), among others (Rahman 2014).

To explain the development of hereditary cancerous retinoblastoma, Alfred Knudson proposed the “2 mutation” model of tumor formation in 1971. According to his insightful model, individuals with hereditary retinoblastoma are at increased risk of tumor formation because they carry an altered copy of a growth-regulating gene (the first mutation) in the germline; that is, in non-cancerous cells. Knudson proposed that if the remaining copy of the gene were to undergo inactivation in a susceptible cell (i.e. the second mutation), then that cell would be prone to tumor formation. Since every cell in an individual with hereditary retinoblastoma carries the first mutation, cancers are more likely to occur at a younger age and in more places . Knudson's prediction was confirmed in 1986 with the identification of the RB1 retinoblastoma gene as the first cancer susceptibility gene. In the decades that

followed this seminal discovery, numerous other cancer susceptibility genes consistent with Knudson's model were identified, including NF1 in neurofibromatosis type 1 (NF1), APC in familial adenomatous polyposis (FAP), TP53 in LFS and BRCA1 and BRCA2 in hereditary breast and ovarian cancer. With the advent of high-throughput sequencing approaches, more cancer predisposing genes are being discovered, and now over 100 different genes and associated syndromes have been identified (Rahman 2014).

Notably, current large-scale sequencing studies reveal that at least 5-12% of all cancer patients have cancer-predisposing germline mutations (Parsons et al. 2016).

The majority of cancer susceptibility genes code for tumor suppressors, proteins that inhibit cell growth by inhibiting cell cycle progression, promoting apoptosis, inducing senescence and/or stimulating differentiation. Tumor suppressors also play an essential role in detecting DNA damage and promoting DNA repair. More rarely, cancer susceptibility is conferred by the presence of activating mutations in growth-promoting oncogenes, including those encoding receptor tyrosine kinases and other intracellular signaling proteins. Whatever the mechanism, mutations in these genes alter normal growth control and thus increase the risk of cancer.

1. Acute leukemia :

Leukemia is a malignant disease involving the excessive production of immature or abnormal leukocytes, which stops the production of normal blood cells and leads to symptoms associated with cytopenias. Malignant transformation usually occurs in a pluripotent stem cell or progenitor with more limited self-renewal capacity. Abnormal proliferation, clonal expansion and decreased apoptosis lead to a depletion of normal blood elements and/or eventual passage of malignant cells into the blood (Kurtin SE et al ., 2011).

Acute leukemia (ALL) is a group of hematological malignancies characterized by clonal proliferation in the bone marrow of blood cell precursors blocked at an advanced stage of differentiation, forming blasts and leading to a significant reduction in normal hematopoiesis. This inability of the normal cell to differentiate, responding to normal physiological stimuli, renders it malignant. These blast cells pass into the bloodstream and invade the spleen and lymph nodes, progressing rapidly and putting patients' lives at risk in the short term if left untreated (it starts suddenly and develops in a matter of days or weeks).

There are two main types: **acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL)** (Diane L ; Gauthier M. ,2014).

2. Acute myeloid leukemia (AML)

Acute myeloid leukemia comprises a number of subtypes and precursor neoplasias that differ from one another in morphology, immunophenotype, cytochemistry and genetic abnormalities, all of which have important implications for prognosis and treatment. Seven classes are described in the WHO classification:

- Acute myeloid leukemia with recurrent genetic abnormalities
- Acute myeloid leukemia with myelodysplastic changes (AML-MRC)
- Treatment-related acute myeloid leukemia (t-AML or t-LMA)
- Acute myeloid leukemia, not otherwise specified (NOS)
- Myeloid sarcoma
- Myeloid proliferations associated with Down syndrome
- Plasmacytoid dendritic blast cell neoplasia (WHO . ,2016)

The morphological criteria of the old Franco-Americano-British (FAB) classification system are used for subtypes not otherwise specified (Table II). Acute promyelocytic leukemia is a subtype of acute myeloid leukemia due to recurrent genetic abnormalities. Acute promyelocytic leukemia is a particular subtype, accounting for 10-15% of acute myeloid leukemias, affecting a rather young age group (median age 31 years), with a higher incidence in certain populations (Hispanics). Patients frequently present with coagulation disorders (Robert PG., 2020).

Acute myeloid leukemia (also known as myeloblastic leukemia, acute myelocytic leukemia, acute myelogenous leukemia, acute granular leukemia and acute nonlymphoblastic leukemia LANL) originates in abnormal myeloid stem cells, these myeloid stem cells usually become an immature type of white blood cell called myeloblasts (or myeloid blasts).

The myeloblasts in AML are abnormal and do not develop into healthy white blood cells. Sometimes, too many stem cells become abnormal red blood cells or platelets. These abnormal white blood cells, red blood cells or platelets are also called leukemic cells or blasts. Leukemia cells can accumulate in the bone marrow and blood, leaving less room for

healthy white blood cells, red blood cells and platelets. When this happens, infection, anemia or easy bleeding can occur. Leukemia cells can spread outside the bloodstream to other parts of the body, including the central nervous system (brain and spinal cord), skin and gums (Robert PG., 2020).

3. <u>Myelodysplastic syndrome :</u>

- **Myelodysplastic syndromes** (MDS) refer to a group of diseases of **the bone marrow**, the tissue that makes blood cells, affecting the **myeloid** lineages, i.e. red blood cells, platelets and certain types of white blood cells, the polynuclear cells. These different cell types circulating in our blood are the result of a series of differentiation and maturation stages taking place in the bone marrow. From the same precursor, or stem cell, this process results in either a red blood cell, a platelet or a **polynuclear** white blood cell. In the course of myelodysplasia, malformations, deformations and impaired function of blood and bone marrow cells of all three lineages are variably observed.

In the blood, there is a quantitative deficit (cytopenias) affecting the red line (anemia), the polynuclear line (neutropenia) and the platelet line (thrombocytopenia). On the contrary, the marrow is rich in morphologically and functionally abnormal cells. The causes of myelodysplastic syndromes are not exactly known. However, certain risk factors have been identified. These include age, smoking and exposure to certain toxic substances such as solvents and pesticides.

Forms secondary to chemotherapy and/or irradiation have also been described. Myelodysplastic syndromes mainly affect people over the age of 65 (4 cases per 100,000 inhabitants per year in France under the age of 60, and 70 cases per 100,000 inhabitants per year over the age of 70).

- **<u>Chronic myeloproliferative syndrome :</u>**

This is a group of diseases characterized by the clonal and malignant proliferation of one or more myeloid cell lines. It is a pathology of the hematopoietic stem cell. The constant feature is hyperplasia of one or more myeloid cell lines, with terminal differentiation of the cells. These are chronic diseases, but their terminal evolution may (not necessarily) take the form

of transformation into acute leukemia. These diseases are currently incurable, except when an allograft is successfully performed. Secondary hyperplasia of the marrow's fibroblastic populations is very often observed, leading to progression to medullary fibrosis. Extra-medullary hematopoiesis is often responsible for myeloid metaplasia (liver, spleen).

- **Chronic myeloid leukemia :**

Chronic myeloproliferative syndrome characterized by a predominant proliferation of the granular lineage associated with a specific cytogenetic anomaly, the t(9 ;22) translocation, also known as the Philadelphia chromosome.

The etiology is unknown, but some cases are secondary to exposure to benzene or ionizing radiation. In these cases, the disease is recognized as an occupational illness. Molecularly speaking, translocation (9;22)(q34;q11) displaces the proto-oncogene

C-ABL from chromosome 9 to chromosome 22, close to a breakpoint called BCR. The hybrid gene, called BCR-ABL, is transcribed as RNA and translated into a fusion protein. This highly unusual molecular anomaly :

- plays a key role in the genesis of the disease, as it confers a proliferative advantage on cells in which this protein is expressed, which seems to be at the origin of the disease; CML is a model for the study of oncogenesis phenomena;
- can be detected with great sensitivity by PCR, enabling the detection of a leukemic cell among 106 normal cells;
- has led to the development of treatments targeting this anomaly, revolutionizing patient treatment and prognosis.

Finally, this cytogenetic anomaly is present in all nucleated hematopoietic cells derived from the malignant clone, including lymphocytes. It will enable us to distinguish pathological from normal bone marrow progenitor populations. Response to treatment can be assessed by cytogenetics and molecular biology.

Chapter V
Solid Tumors

1. **Lung cancer**

Lung cancer can be histologically and clinically categorized into small-cell lung cancer (SCLC), a subtype with a neuroendocrine phenotype, and non–small–cell lung cancer (NSCLC), which includes adenocarcinoma, squamous-cell carcinoma, and large-cell carcinoma. The cellular substrates for molecular analysis have included normal-appearing respiratory epithelial cells, preneoplastic epithelial lesions, invasive cancers, and metastasis to other organs, as well as long-established tumor cell lines.

Indeed, the establishment of lung cancer cell lines allowed initial identification of the multiple and often shared cytogenetic abnormalities in lung cancer, including gain or loss of chromosomal regions. Modern techniques allow us to detect much smaller genetic and even epigenetic alterations acquired in the genome of these cell lines, findings that can then be confirmed in primary lung cancer specimens, thereby adjusting for the cellular derangements due solely to repeated passage and culture of tumor cells in vitro.

It is becoming clear that the genetic changes acquired by lung cancers are not only multiple but also complex and heterogeneous both in chronology and mechanistic pathways.

Cancer cells may harbor a homogeneously amplified region of chromosome 8q, consistent with activation of the protooncogene MYC through copy number amplification. In addition, there may be frequent loss of heterozygosity at chromosomal loci. Such loss of heterozygosity indicates one of the two “hits” that are generally required to inactivate a tumor suppressor gene (TSG), 17p for p53, 9p21 for p14ARF and p16INK4a, 13q14 for RB, and multiple loci of 3p for FHIT, RASSF1A, and/or other unidentified genes. There are also emerging data on the molecular lesions that are specific to one of the two major lung cancer subtypes and those that are common to both. Some genes are targeted for both subtypes (and other solid human cancers), such as mutations of p53. Others can be relatively specific to a subtype and may play a role in its differentiation. For example, SCLC features more frequent alteration in myc activation and RB inactivation than NSCLC, whereas NSCLC has more in ras activation and p16INK4a inactivation. As to mechanisms of molecular damage, it has long been known that classical molecular genetic changes reflect the activation of protooncogenes and inactivation of TSGs. More recent is the appreciation that abnormalities of these individual gene products, often in combinatorial complexes, partner to underpin intricate intracellular signaling networks, which become deranged in cancer cells. So it is

often advantageous to think of lung neoplasia in the setting of classic "hallmarks of cancer," which include self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (Hanahan et Weinberg 2011), with eachcomponent deranged by abnormalities in different gene products.

2. Pancreatic carcinoma

A small proportion, approximately 5-10% of the familial aggregation of pancreatic cancer is thought to be explained by hereditary cancer syndromes and inherited forms of pancreatitis, caused by rare high-risk inherited mutations (Goldstein et al. 2006). Multiple genes have been identified as carrying mutations that increase the risk of pancreatic cancer, most often within multi-cancer familial cancer syndromes, but also in patients with inherited mutations that cause hereditary pancreatitis.

Here are some examples of the syndromes that can cause pancreatic cancer :

- **Peutz-Jeghers syndrome** is a rare autosomal dominant disease characterized by melanocytic macules of lips, buccal mucosa and digits, and benign intestinal polyps with a greatly increased risk of multiple malignancies that include gastrointestinal, breast, and gynecological cancers (S. E. Korsse, Peppelenbosch, et van Veelen 2013). Mutations in the ***STK11*** gene (chr19p13.3) which encodes the tumor suppressor serine/threonine protein kinase STK11 cause Peutz-Jeghers syndrome. They are associated with a high risk of pancreatic cancer (relative risk estimates range from 76-132) in studies conducted in the U.S. and Europe (Susanne E. Korsse et al. 2013). ***STK11*** regulates diverse processes, such as cell growth, cell polarity, energy metabolism, and apoptosis, mainly via the regulation of AMPK/mTOR signaling (S. E. Korsse, Peppelenbosch, et van Veelen 2013).

- **Familial atypical multiple mole and melanoma syndrome (FAMMM)** Inactivating mutations in the ***CDKN2A*** tumor suppressor gene (chr9p21.3) are associated with familial melanoma, an autosomal dominantly inherited cancer syndrome termed FAMMM (Foulkes et al. 1997).

Other cancers, including pancreatic cancer, are seen at a higher-than-expected frequency in a subset of FAMMM families (Rutter et al. 2004). The ***CDKN2A*** gene encodes cyclin-dependent kinase inhibitor 2A (also called p16), an important cell cycle regulator that negatively regulates cell proliferation (Foulkes et al. 1997). Among pancreatic cancer cases, either unselected or selected for positive pancreatic cancer family history, 0.6-3.3% have been described to carry deleterious germline mutations in ***CDKN2A***, respectively (Zhen et al. 2015). Specific ***CDKN2A*** mutations appear to be associated with a high frequency of pancreatic cancer. An example is the Dutch Leiden founder mutation (19 bp deletion in ***CDKN2A***) which is associated with an estimated 48-fold increased risk of pancreatic cancer (de Snoo et al. 2008).

- **Hereditary nonpolyposis colorectal cancer (HNPCC)**, or **Lynch syndrome**, is characterized by germline mutations in DNA mismatch repair (MMR) genes that include ***MLH1*** (chr3p22.2), ***MSH2*** (chr2p21), ***MSH6*** (chr2p16.3) and ***PMS2*** (chr7p22.1). Bi-allelic loss of an MMR gene leads to genomic instability which is often manifested by microsatellite instability in colorectal tumors. Mutation carriers have an increased risk of multiple cancer types, most notably colorectal and endometrial cancer, but also pancreatic cancer (Kastrinos et Stoffel 2014). A large study that included 147 families (over 6,000 individuals) with mutations in MMR genes, noted at least one case of pancreatic cancer in ~21% of families. The cumulative risk of pancreatic cancer up to age 70 was 3.68% in this study, or close to a 9-fold increased risk as compared to the general population (Kastrinos et al. 2009). **Familial adenomatous polyposis** (FAP) syndrome is characterized by numerous (often thousands) colorectal polyps and a greatly increased risk of colon cancer. This syndrome is caused by mutations in the ***APC*** tumor suppressor gene on chr5q22.2, which encodes a negative regulator of the WNT signaling pathway (Dalavi et al. 2015). Pancreatic cancer has been observed at a higher-than-expected frequency in affected individuals with an estimated relative risk (RR) of 4.46 (Groen et al. 2008).

3. Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver and accounts for 90 % of hepatic cancers. Common risk factors for HCC are chronic inflammatory infections caused due to hepatitis B and hepatitis C viruses. These infections lead to cirrhosis and are responsible for making HCC the most recurrent cancer worldwide.

Other risk factors associated with HCC are hereditary hemochromatosis, steatohepatitis related to obesity, alcohol consumption, and diabetes (Marengo, Rosso, et Bugianesi 2016). Additionally, certain rare diseases increase HCC risk including alpha1-antitrypsin deficiency, Wilson disease, and tyrosinemia. Epigenetic alterations are known to result in changes in gene expression and have been shown to trigger HCC (Erkekoglu et al. 2017). These changes confer survival advantages for cancer cells which is a hallmark of HCC. The liver constantly adapts to variations in environmental conditions related to dietary xenobiotics, viral infections, and alterations in the microbiota (Wilson, Mann, et Borthwick 2017). The etiology of HCC is closely related to environmental factors also indicates that epigenetic aberrations can contribute to the initiation and promotion of HCC (Erkekoglu et al. 2017). These environmental stresses lead to alterations in DNA methylation, acetylation, chromatin modifications, long noncoding RNAs (lncRNAs), and miRNA bringing about changes in the hepatic epigenome. Accumulation of these epigenetic modifications and alterations eventually causes dysregulated expression of tumor suppressor genes and oncogenes manifested in carcinogenesis, progression, and metastasis of HCC (Cheishvili, Boureau, et Szyf 2015). The advancements in the next-generation sequencing have led to an in-depth understanding of genomics and epigenomics in HCC. The public genomic databases including the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA) have made it much easier for the research community to better understand epigenetic modifications and genetic alterations. In this article, we describe the basic epigenetic mechanisms and the role of dysregulated epigenetic alterations in promoting HCC.

The epigenetic modifiers play a crucial role in modifying the epigenome via directly introducing DNA methylation, altering the chromatin marks, or post-translational modifications of chromatin. The epigenetic modifiers include epigenetic writers, readers, and erasers. The epigenetic writers include enzymes that introduce covalent alterations to histones and DNA. The writers are DNMTs, HATs, histone lysine methyltransferases (HMTs/KMTs),

and serine-threonine and tyrosine kinases (Fig 15). The epigenetic erasers are the collective enzymes that remove the reversible epigenetic marks. The erasers include the DNA methylation system, lysine-specific demethylase (LSD), histone deacetylases (HDACs), protein phosphatases (PPs), and histone demethylases (HDMS) (Fig. 16). Epigenetic readers recognize the functional modifications of epigenetic marks placed on the DNA and histones that possess binding domains for covalent modifications. The epigenetic readers include methyl CpG binding proteins (MBPs), histone acetylation readers (bromodomain), and histone methylation readers (chromodomain), as shown in (Fig.17). These epigenetic marks are found as multimeric complexes together and enable modulation of chromatin conformation via dynamic integration signaling (Biswas et Rao 2018).

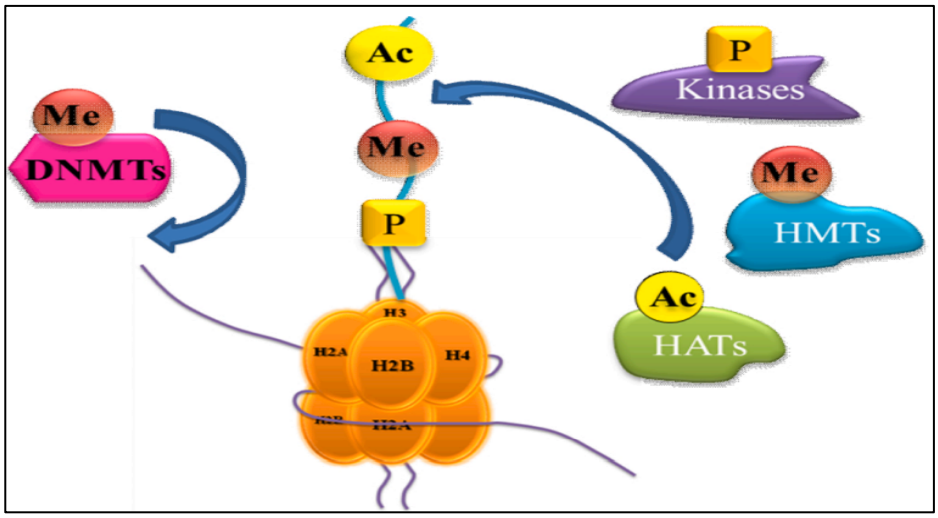


Figure 15 Epigenetic modifiers.Epigenetic writers introduce covalent alterations to histones and DNA. Epigenetic writers include epigenetic enzymes such as DNA methyltransferases (DNMTs), histone acetyltransferases (HATs), histone lysine methyltransferase (HMT/KMTs), and serine-threonine and tyrosine kinases. Methyl group (Me), acetyl group (Ac), phosphorylation (P). H represents histone type. DNA contains 5-methylcytosine (5-mc) that encourages epigenomic studies. Ten-eleven translocation methylcytosine deoxygenase (TET) oxidizes 5mc to produce 5 fc and 5caC, thus silencing the tumor suppressor genes. TDG excises 5fC/5caC as a process of base excision process (BER) to generate a basic site. BER excises a basic site and replaces nucleotide (cytosine) using deoxycytidine triphosphate (dCTP).

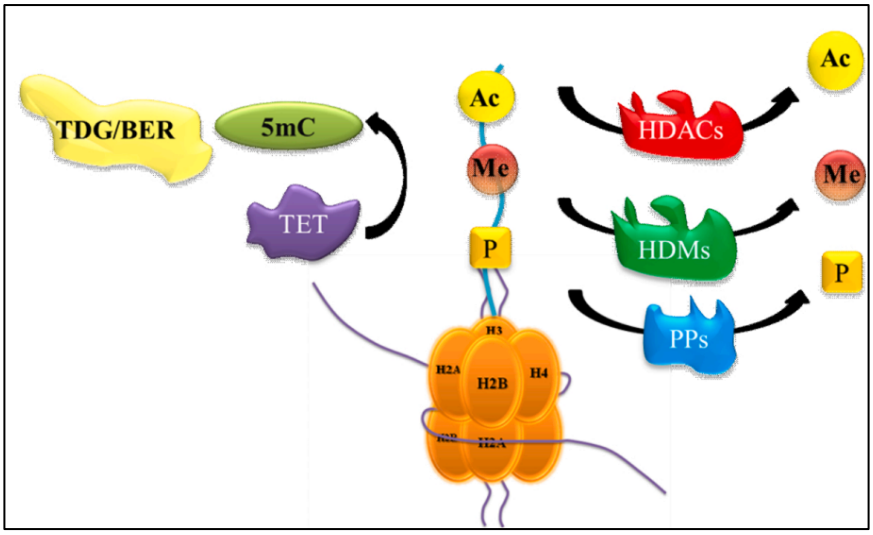


Figure 16 Epigenetic modifiers Epigenetic erasers include enzymes that remove reversible epigenetic marks. DNA methylation system, histone deacetylases (HDACs), lysine-specific demethylase (LSD), histone demethylases (HDMS), and protein phosphatases (PPs) are epigenetic erasers. Methyl group (Me), acetyl group (Ac), phosphorylation (P). H represents histone type.

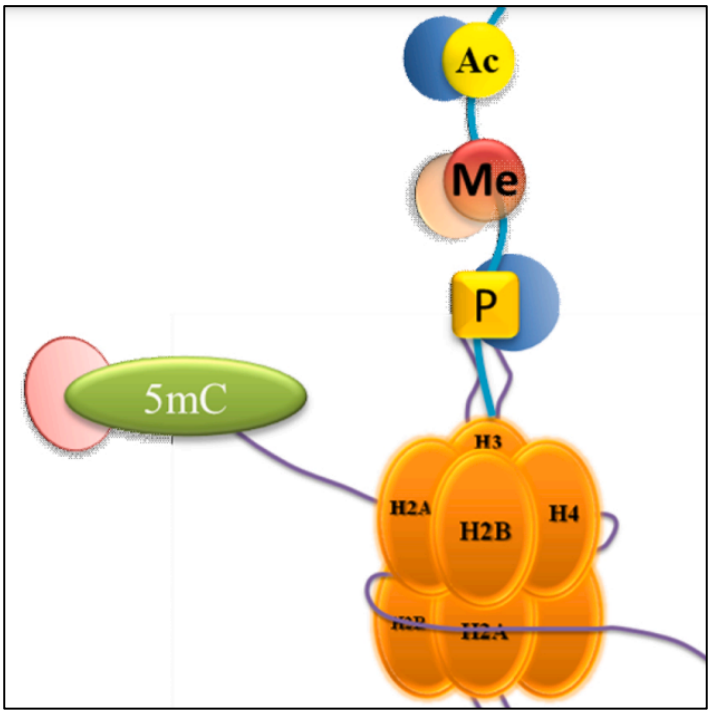


Figure 17 Epigenetic modifiers Epigenetic readers identify the functional alterations of epigenetic marks present on DNA and histone that have binding domains for covalent modifications. Methyl CpG binding protein (MBPs), histone methylation (chromodomain), and histone acetylation (bromodomain) readers include epigenetic readers. Methyl group (Me), acetyl group (Ac), phosphorylation (P). H represents histone type. 5mC (methylated cytosine) is the 5th base (cytosine) of methylated DNA.

Chapter VI : Cancer treatments : Special therapeutic procedures

1. Hematopoietic stem cell technology

The cellular potential of hematopoietic stem cells (HSCs)(fig 18) has been traditionally defined by transplanting donor cells (or a single cell) into recipients that are preconditioned by lethal irradiation and therefore devoid of a functional endogenous hematopoietic system. This assay has long been the gold standard for functional HSCs. The first ***in vivo*** evidence for the existence of HSCs, in 1961, was based on the rescue of lethally irradiated recipient mice by bone marrow transplantation, followed by observing hematopoietic colonies in the spleens of recipients (Till and Mc, 1961). Thereafter, scientists were interested in developing methods to purify HSCs from bone marrow to better understand their function and molecular regulatory networks. Separation of HSCs became possible with the utilization of antibodies and fluorescence-activated cell sorting (FACS). Weissman and colleagues first described HSC-enriched cells using the combination of several surface markers in 1988 (Spangrude et al., 1988). Since then, different groups have put great effort into identifying more surface markers to further purify HSCs. To date, CD34, Sca-1, c-Kit, the signaling lymphocyte activation molecule (SLAM) markers, etc. are still commonly used to isolate HSCs in

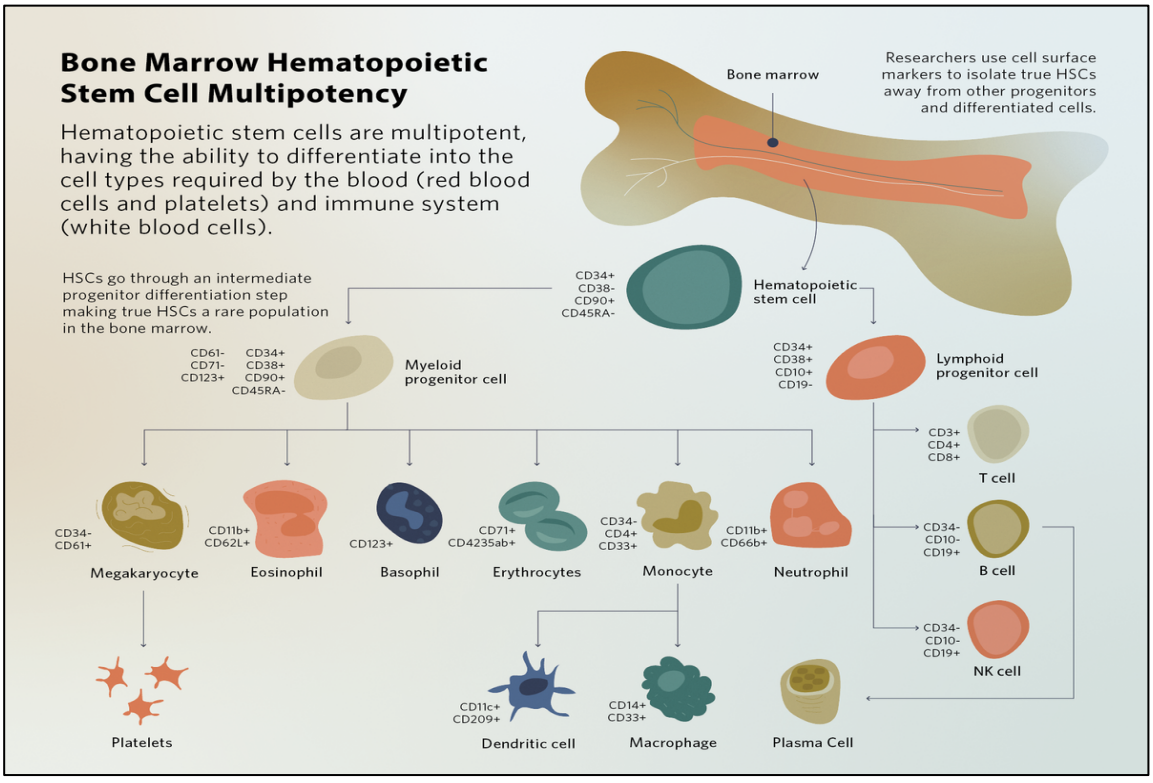


Figure 18 Bone marrow hematopoietic stem cell multipotency

different labs (Ikuta and Weissman, 1992; Okada et al., 1992; Osawa et al., 1996; Kiel et al., 2005; Oguro et al., 2013). Since similar approaches can be used to identify multi- and unipotent progenitors, different progenitor populations were also isolated based on surface markers (Kondo et al., 1997; Akashi et al., 2000; Adolfsson et al., 2005; Wilson et al., 2008; Pietras et al., 2015).

Through transplantation and colony assay, HSCs have been defined based on two essential properties, self-renewal and multipotent differentiation, which can produce cells of all blood lineages (Morrison et al., 1995; Orkin, 2000; Reya et al., 2001; Dick, 2003; Reya, 2003). By contrast, progenitors have been defined by the absence of self-renewal and restricted lineage differentiation capacities. To better illustrate the relationship between an HSC and its progenies and the stepwise differentiation process, the immunophenotype-based tree-like hierarchy model was largely established by Weissman's group (Kondo et al., 1997; Morrison et al., 1997; Akashi et al., 2000; Manz et al., 2002). In this classical model, HSCs can be divided into two subpopulations according to their CD34 expression: $CD34^-$ long-term (LT)-HSCs and $CD34^+$ short-term (ST)-HSCs. LT-HSCs are a rare, quiescent population in the bone marrow and have full long-term (> 3~4 months) reconstitution capacity, whereas ST-HSCs only have a short-term (mostly < 1 month) reconstitution ability. LT-HSCs differentiate into ST-HSCs, and ST-HSCs differentiate into multipotent progenitors (MPPs), which have no detectable self-renewal ability (Yang et al., 2005). The first bifurcation occurs between the common myeloid progenitors (CMPs, with myeloid, erythroid, and megakaryocytic potential) and common lymphoid progenitors (CLPs, with only lymphoid potential), which are derived from MPPs. The second branch point at CMPs segregates bipotent granulocyte-macrophage (GMPs) and megakaryocyte-erythrocyte progenitors (MEPs). CLPs further form T, B, NK, and dendritic cells, while GMPs differentiate into granulocytes/monocytes and MEPs generate megakaryocytes/erythrocytes. All these populations form a tree-like and balanced hierarchy model, within which key transcription factors (TFs) and cytokines precisely conduct the stepwise differentiation of HSCs to mature blood cells (Zhu and Emerson, 2002; Robb, 2007; Metcalf, 2008; Zhang and Lodish, 2008; Seita and Weissman, 2010).

2. Autologous hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation is a well-established multistep procedure designed to replace the blood and lymphoid systems of a patient with a new one derived from hematopoietic stem cells (HSCs). HSCs can be collected from either a healthy donor (allogeneic transplantation) or the patient (autologous transplantation). The procedure has been used extensively in the past 50 years for the treatment of aggressive hematological malignancies, such as leukemia and lymphoma (Appelbaum 2007). Allogeneic transplantation is most frequently used for malignant indications but carries the risk of graft-versus-host disease (GVHD), which increases transplant-related mortality. The risk is partially offset by a lower incidence of leukemia relapse than that observed with autologous transplantation, an advantage that is attributed to a graft-versus-leukemia effect. The prevention of relapse using graft-versus-host effects has also been reported in autoimmune disease (graft-versus-autoimmunity) (Hinterberger, Hinterberger-Fischer, et Marmont 2002), but the risk of transplant-related mortality from allogeneic transplantation is generally considered unacceptable in non-neoplastic diseases.

The AHSCT procedure comprises four main steps: HSC mobilization, HSC harvesting, ablative conditioning, and HSC re-infusion or ‘transplantation’ (FIG. 19). Initially, HSCs were obtained by aspiration of the bone marrow, but are increasingly harvested from peripheral blood after so-called mobilization. HSC mobilization involves the administration of granulocyte colony-stimulating factor (G-CSF), either alone or with cytotoxic chemotherapy, such as cyclophosphamide. HSCs that have been mobilized are then harvested from peripheral blood by leukoapheresis. The HSCs are cryopreserved and stored frozen until the patient is ready for transplantation. Before transplantation, ablation of the haemato-lymphopoietic system is achieved with high-dose chemotherapy (or chemoradiotherapy when associated with total body irradiation, which is no longer used for MS but is for other indications); this stage is known as the preparative or conditioning regimen. Immediately after completion of the conditioning regimen, patients develop pancytopenia and a transient bone marrow aplasia, and intravenous infusion of the stored HSCs (transplantation) is required to enable marrow repopulation, recovery of hematopoiesis, and immune reconstitution. The duration of HSC mobilization and leukapheresis is 5–15 days, depending

on the protocols employed, and can be performed in daycare or with a short hospital admission. Conditioning and transplantation require hospital inpatient admission to enable close monitoring and supportive care. Ablative conditioning therapy generally starts at least 2–4 weeks after completion of HSC harvesting, but should not be delayed if it is safe to proceed. Patients are usually admitted for 3 weeks (Saccardi et Gualandi 2008).

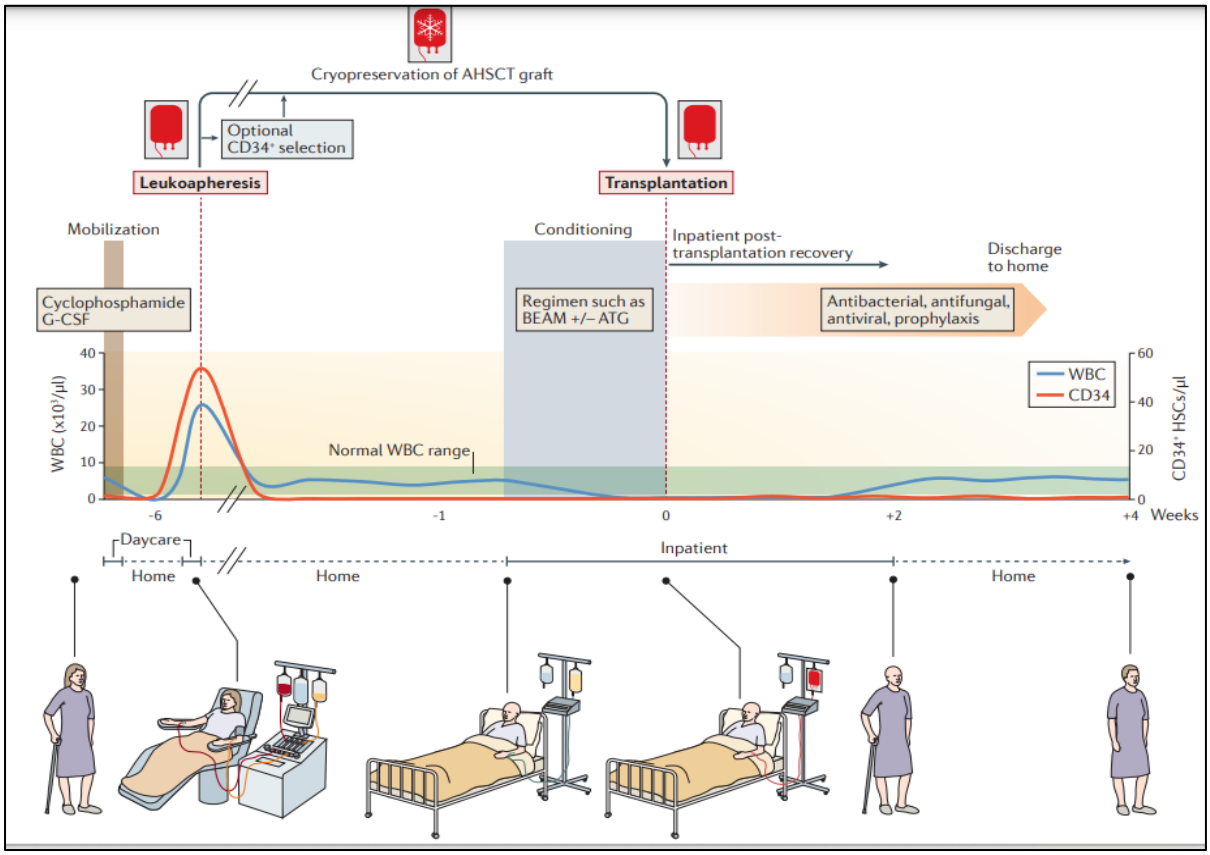
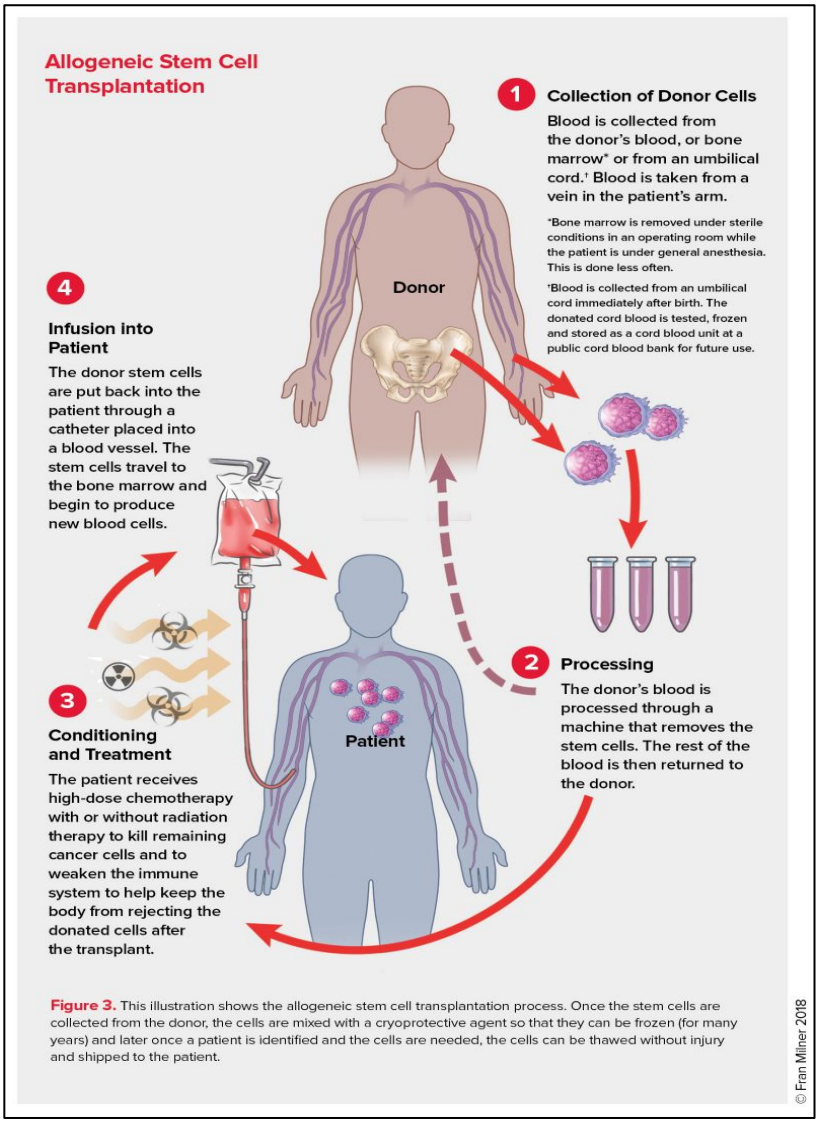


Figure 19 Outline of the AHSCT procedure Key steps of the procedure, drugs administered white blood cell (WBC) and CD34+ hematopoietic stem cell (HSC) counts, and the patient's condition and disposition are arranged from top to bottom. The indicative timescale covers 10 weeks. The procedure starts with the mobilization of HSCs from the bone marrow by injection of cyclophosphamide intravenously and granulocyte-colony stimulating factor (G-CSF) subcutaneously. The autologous graft harvested from the peripheral blood by leukapheresis, which can undergo CD34 selection to enrich HSCs or can be unmanipulated, is cryopreserved for subsequent use. Ablation of the immune and, to a variable extent, the myeloid system is most commonly achieved by high-dose conditioning with a combination of cytotoxic drugs. The autologous hematopoietic graft is then reinfused (transplantation), and anti-thymocyte globulin (ATG) is often administered with the conditioning regimen to deplete T cells; owing to the long half-life of ATG, it will also deplete and prevent the engraftment of any T cells present in the autologous graft (in vivo graft T cell depletion). Different levels of supportive care are required during the procedure; the conditioning, transplantation, and in vivo T cell depletion steps require inpatient admission until the patient has recovered from neutropenia and the management of any complications is complete. AHSCT, autologous hematopoietic stem cell transplantation; BEAM, bis-chloroethyl nitrosourea, etoposide, cytosine arabinoside, and melphalan.

3. Allogeneic hematopoietic stem cell transplantation

Allogeneic stem cell transplantation involves the use of stem cells from someone other than the patient. The donated stem cells can come from either a person related or not related to the patient. Before beginning an allogeneic SCT, the patient receives a conditioning treatment that consists of either chemotherapy or radiation. Some patients receive both. Conditioning treatment is given to destroy any remaining cancer cells in the body. It weakens the patient's immune system so that the donor cells are not rejected. Conditioning treatment allows the new cells to move through the bloodstream to the patient's bone marrow, where the donor cells begin to grow and produce new blood cells, including red blood cells, white blood cells, and platelets. This process is called "engraftment." For some types of blood cancers, an allogeneic SCT may work directly to destroy cancer cells. This is called the graft-versus-tumor (GVT) effect. The GVT effect happens when white blood cells from the donor (the "graft") identify any remaining cancer cells (the "tumor") in the patient as foreign and attack them. For some patients, GVT is crucial for the effectiveness of their treatment. It can help prevent their cancer from coming back. This benefit can only occur in allogeneic SCT. It does not occur in an autologous SCT. Allogeneic SCT is often used to treat



blood cancers such as leukemia, myelodysplastic syndromes, and myeloproliferative neoplasms.

Once it is determined that allogeneic SCT is a treatment option for a patient, the patient's doctor will begin to search for a suitable donor. For most patients, a close match is important because it improves the chances for a successful transplant by:

- Helping the donor stem cells engraft (grow and make new blood cells in the patient's body)
- Reducing the risks of complications

HLA Matching. People have different sets of proteins or markers called human leukocyte antigens (HLAs) on the surface of most of their cells. They make up a person's tissue type, which varies from person to person. Blood tests of both the patient and potential donor are done to determine if there is an HLA match. There are many HLA markers. Individuals inherit half of their HLA markers from their mothers and half from their fathers, so most often the ideal donor is a patient's sibling (who has inherited the same HLA markers). On average, a person has one chance in four of having the same HLA type as his or her sibling, but many patients do not have a sibling with the same tissue type. For those patients who do not have a matched family donor, an unrelated donor may be found through a volunteer donor registry.

Mismatched Unrelated Donor Transplantation. Your doctor will try to match 10 to 12 HLA markers to lower the risk of graft-versus-host disease (see below). In recent years, advances in medicine have allowed for the use of stem cell donors who are mismatched, meaning that not all 10 or 12 markers are a perfect match. The use of medications following transplant allows for mismatched donors while still lowering the risk of graft-versus-host disease.

Haploidentical Transplantation. To increase the number of potential donors, some transplant centers have begun to perform half-match (haploidentical) transplants for patients who cannot find a closely matched HLA donor. Often, a healthy first-degree relative (a parent, sibling, or child) can be a half-match donor and donate stem cells. Because a child receives half of their HLA markers from a parent, a biological child, and their parent will

always be a half match, while there is only a 50 percent chance of a sibling being a half match. As a result, most individuals will have a suitable related haploidentical donor.

Cord Blood Transplantation. Cord blood is blood taken from the umbilical cords of newborn babies. Cord blood may be an option for patients without a well-matched donor. Unfortunately, cord blood units tend to contain fewer stem cells and may be difficult to use in people with larger body sizes. Cord blood transplant patients also have an increased risk of graft failure. However, cord blood is available much more quickly (potentially within 2 to 4 weeks), while it may take a month or more to obtain matched unrelated donor grafts. Another advantage of cord blood transplants is that cord blood may require a lower level of HLA matching between the donor and recipient.

Possible Complications

One complication of allogeneic transplantation is that the patient's body—despite the treatment to suppress the immune system—may reject the donated stem cells before they can engraft in the bone marrow. The patient's immune cells may see the donor's cells as foreign and destroy them.

Graft-versus-host disease (GVHD) is a common but potentially serious complication of standard allogeneic and reduced-intensity allogeneic SCT. Sometimes, the cells from the donor (graft) sense that the healthy cells of the patient (host) are foreign and attack and damage the patient's normal cells. GVHD can be mild, moderate or severe. In some cases, it can be life-threatening.

A close HLA match between the donor and patient helps to lower the risk of GVHD. Some medications help prevent GVHD. Even with a close HLA match and medication, some people still get GVHD.

Allogeneic stem cell transplants for patients who are older or have overall poor health are relatively uncommon. This is because the pre-transplant conditioning therapy is generally not well tolerated by such patients, especially those with poorly functioning internal organs. However, reduced intensity may be an appropriate treatment option for certain older patients, who have diseases that involve major organs, such as the heart or liver, or who are otherwise not healthy or strong enough to undergo standard allogeneic transplantation.

5. Granulocyte transfusion

The use of granulocyte transfusions in the 1970s and 1980s was limited by the difficulty of collecting sufficient numbers of cells from healthy donors to demonstrate more than a modest success of this therapy in severely neutropenic patients. The availability of centrifuge methods for continuous collection of granulocytes and the use of recombinant human granulocyte colony-stimulating factor (rHuG-CSF) to stimulate donors resulted in an increased interest in granulocyte transfusions since 1995, and studies with granulocytes collected from donors given G-CSF were initiated. These studies have shown that G-CSF increased the average number of cells in granulocyte concentrates to 4·1 × 1010, whereas granulocyte concentrates collected from donors given steroids contained only 1·5–2·5 × 1010 cells. However, the evidence that the provision of granulocytes from G-CSF-stimulated donors is efficacious in clearing infection or prolonging patient survival is limited to that provided by case reports or small uncontrolled series. As a consequence of the use of rHuG-CSF in the conditioning of granulocyte donors, new interest has emerged in granulocyte transfusion therapy.

Granulocyte donors

Before the discovery and development of human growth factors for clinical use, the inability to collect adequate numbers of functional polymorphonuclear leucocytes (PMNL) from healthy donors hindered the development of granulocyte transfusion therapy. Without substantial neutrophilia in the donor, the yield of PMNL obtained by leukapheresis is limited. Corticosteroids conventionally have been used to mobilize PMNL in granulocyte donors. As mentioned previously, this practice allows for the collection of a relatively small number of PMNL (10 × 109 −30 × 109 /leucapheresis), which usually results in only a transient increase in the blood PMNL count when transfused in neutropenic patients (Strauss 1993).

In haematologically normal individuals, administration of G-CSF is safe and well tolerated. After multiple doses of daily G-CSF, most leucocytes in the peripheral blood of the donor remain mature PMNL; however, small numbers of bands, promyelocytes, metamyelocytes, and myelocytes do begin to appear in the circulation with repetitive G-CSF administration (Bensinger et al. 1993). A slight increase in the absolute number of lymphocytes is also observed, but monocyte counts do not change appreciably. After a 5-day treatment with G-CSF, no severe adverse effects were reported in one study (Machida et al. 2000). Moreover,

no long-term adverse events have been reported after the administration of G-CSF to healthy individuals. In a study of 19 donors, blood counts 1 year after the collection of G-CSF-stimulated peripheral blood stem cells (PBSC), and the results of a second mobilization and collection were analyzed (Stroncek et al. 1997). Subjects in this study received a regimen of 2–10 μg of G-CSF/kg/day for 5 days on 2 occasions, separated in time by = 12 months. One year after the administration of G-CSF, blood counts were normal and unchanged, and the yield of PBSC from the second leukapheresis procedure was similar to the yield achieved from the first collection. Several studies have demonstrated that the administration of 300 μg of G-CSF subcutaneously (SC) can elevate the peripheral blood PMNL count 5–6-fold within 12–24 h, compared with a 2–3-fold increase observed after corticosteroid therapy. In addition, recent evidence indicates that the additional administration of corticosteroids significantly increases the level of neutrophilia induced in normal subjects by single-dose G-CSF (Liles et al. 1997). In one study, healthy volunteers were randomly assigned to receive each of the following 5 single-dose regimens: (7) G-CSF, 300 μg given SC; (8) G-CSF, 600 μg SC; (1) dexamethasone, 8 mg given orally; (9) G-CSF, 300 mg SC, plus dexamethasone, 8 mg orally; and (10) G-CSF, 600 μg SC, plus dexamethasone, 8 mg orally (Liles et al. 1997). All five drug regimens induced a rapid neutrophilic response in the subjects, which was evident within 6 h after drug administration and sustained through 24 h. Except for the administration of dexamethasone alone, the maximal PMNL count observed after each regimen occurred at the 12-hour time point. Mobilization of PMNL was greatest after the administration of G-CSF (600 μg) and dexamethasone (8 mg); the PMNL count increased > 12-fold from a mean baseline value of 3594/μl to 43 017/μl at 12 h. All the drug regimens were well tolerated. The most commonly reported side effects were myalgia and arthralgia, followed by headache. These side effects resolved within 24 hours, and no donors requested or required dismissal from the study protocol as a result of side effects. Enhanced PMNL mobilization in donors effectively results in an enhanced yield of PMNL by centrifugation leukapheresis. Regardless of whether G-CSF or corticosteroids are used for donor stimulation, mature PMNL constitutes> 75% of the leucocytes in the granulocyte concentrate. In a recent study, 16 normal subjects received G-CSF (600 μg SC) and dexamethasone (8 mg orally) 12 h before leukapheresis [11]. A mean of 77·4 × 109 PMNL was collected with each leukapheresis. The functional properties of the PMNL remained normal or near normal.

Specifically, the respiratory burst in response to phorbol myristate acetate (PMA), N-formyl-met-leu-phe (FMLP), tumor necrosis factor (TNF)-α, and lipopolysaccharide was assessed in PMNL from venous blood after the administration of G-CSF and dexamethasone before leukapheresis and in PMNL obtained from the concentrate after leukapheresis (Martino, s. d.). In the latter case, PMNL retained the respiratory burst activity in response to all four stimuli. PMNL isolated from venous blood after the administration of G-CSF and dexamethasone were primed to undergo an enhanced respiratory burst in response to either TNF-α or lipopolysaccharide. In the concentrate, the bactericidal capacity against Staphylococcus aureus was normal, and the surface expression of CD11b, CD18, CD14, CD32, and CD64 was increased. In five subjects, PMNL was reinfused 23 hours after collection. The half-life for the infused cells was significantly longer than that of normal blood PMNL (20·3 ± 2·1 h vs. 9·6 ± 1·2 h) [11]. In another study, 78 granulocyte concentrates were collected from 20 donors after stimulation with G-CSF, and functional as well as immunological parameters of granulocytes in donor samples before and after receiving multiple G-CSF stimulation, in granulocyte concentrates and in the patients 8 h after transfusion were analyzed. Granulocyte concentrates contained 52·5 ± 2·42 × 109 cells. Chemotaxis was influenced neither by G-CSF application nor by apheresis. Multiple G-CSF stimulations enhanced oxidative burst and phagocytosis of E. coli in donor granulocytes. These values returned to basal levels in granulocyte concentrates. Expression of granulocytic surface antigens was downregulated after application of G-CSF but returned to normal and in part enhanced values in concentrates. A clinically relevant increase of proinflammatory cytokines could not be detected. Leukotriene (LTB4) production was reduced after the fourth G-CSF stimulation in the donor blood and enhanced in the granulocyte concentrate after apheresis. Results in recipients indicated that changes in granulocyte function noted in concentrates were only transient (Martino, s. d.).

Granulocyte collection

Since the advent of leukapheresis in 1962, several methods have been tested for the collection of PMNL, including filtration leukapheresis, gravity, and intermittent-flow centrifugation (Menitove et Abrams 1987). Currently, most apheresis units employ continuous-flow centrifugation leukapheresis, which selectively removes a leucocyte fraction containing predominantly PMNL and returns the red cells and platelets to the donor. Blood

from one antecubital vein travels to a pump, where a sedimenting agent, usually hydroxyethyl starch with citrated anticoagulant, is added. The use of hydroxyethyl starch, which increases erythrocyte sedimentation by causing rouleaux formation, doubles the efficiency of leukapheresis and significantly increases the number of erythrocytes returned to the donor (Schiffer et al. 1975). Setting the centrifuge apparatus to a higher interface offset improves the collection yield without significant effects on the donor (Adkins et al. 1998). Granulocyte storage In current practice, PMNLs are collected and transfused soon after apheresis. One of the factors contributing to the decline in the activity of stored PMNL is spontaneous apoptosis. PMNL die rapidly via apoptosis in vivo and in vitro, and apoptotic cells demonstrate a reduced ability to degranulate, generate an oxidative burst, or undergo shape changes in response to external stimuli (Liles et Klebanoff 1995). Among its physiological effects, G-CSF significantly decreases the rate of PMNL apoptosis in vitro, thereby extending the functional life span of PMNL in culture (F. Colotta et al. 1992).

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