Ministry of Higher Education and Scientific Research

A MIRA-BEJAIA University

Faculty of Natural and Life Sciences

Department of Physico-Chemical Biology

Oncology

Course handout intended for first-year Master's students in the Genetic program.

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2024/2025

"La persévérance est la clé du succès."

Oncology

by

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« Comprendre le cancer, c'est comme déchiffrer un langage complexe ; chaque cellule, chaque mutation, chaque interaction nous révèle un peu plus sur ce vice insidieux qui menace tant de vies. Seule cette compréhension peut nous éclairer dans notre lutte contre cette maladie. »

Liste des abréviations

AIF : Apoptosis-Inducing Factor

AKT/PKB : Protein Kinase B

APC : Adenomatous Polyposis Coli

BAX : Bcl-2-Associated X Protein

Bcl-2 : B-Cell Lymphoma 2

BH3 : Bcl-2 Homology Domain 3

CAM : Cell Adhesion Molecule

CARD : Caspase Recruitment Domain

CD44 : Cluster of Differentiation 44

CD95 : Cluster of Differentiation 95 (Fas Receptor)

CDK : Cyclin-Dependent Kinase

CKI : Cyclin-Dependent Kinase Inhibitor

Cdk1 : Cyclin-Dependent Kinase 1

Cdk4 : Cyclin-Dependent Kinase 4

Cdk6 : Cyclin-Dependent Kinase 6

Cdk7 : Cyclin-Dependent Kinase 7

CIP/KIP : Cyclin Inhibitory Proteins / Kinase Inhibitory Proteins

CRD : Carbohydrate Recognition Domain

DED : Death Effector Domain

DISC : Death-Inducing Signaling Complex

Drp-1 : Dynamin-Related Protein 1

E2F : E2 Promoter Binding Factor

ECM : Extracellular Matrix

FADD : Fas-Associated Death Domain

FAK : Focal Adhesion Kinase

GrA : Granzyme A

GrB : Granzyme B

HER2 : Human Epidermal Growth Factor Receptor 2

ICAM : Intercellular Adhesion Molecule

IAP : Inhibitor of Apoptosis Protein

ILK : Integrin-Linked Kinase

JAK/STAT : Janus Kinase / Signal Transducer and Activator of Transcription

Mdm2 : Mouse Double Minute 2 Homolog

MAPK : Mitogen-Activated Protein Kinase

MMP : Matrix Metalloproteinase

MPF : Mitosis Promoting Factor

NCAM : Neural Cell Adhesion Molecule

NM23 : Non-Metastatic Gene 23

PIDD : p53-Induced Protein with a Death Domain

PI3K : Phosphoinositide 3-Kinase

PSTAIRE : Amino Acid Motif in Cyclin-Dependent Kinase

Rb : Retinoblastoma Protein

RAP1 : Repressor/Activator Protein 1

SAM : Substrate Adhesion Molecule

Smac : Second Mitochondria-Derived Activator of Caspase

TERC : Telomerase RNA Component

TERT : Telomerase Reverse Transcriptase

TIN2 : TRF1-Interacting Nuclear Protein 2

TNF : Tumor Necrosis Factor

TNFR : Tumor Necrosis Factor Receptor

TRADD : TNF Receptor-Associated Death Domain

TRAIL : TNF-Related Apoptosis-Inducing Ligand

TRF : Telomeric Repeat-Binding Factor

TRF1 : Telomeric Repeat-Binding Factor 1

TRF2 : Telomeric Repeat-Binding Factor 2

uPA : Urokinase Plasminogen Activator

uPAR : Urokinase Plasminogen Activator Receptor

VEGF : Vascular Endothelial Growth Factor

VDAC : Voltage-Dependent Anion Channel

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Introduction

Cancer is one of the most common pathologies nowadays, considered the “disease of the century”. This pathology is characterized by uncontrolled and excessive multiplication due to a loss of the standard regulatory mechanisms of the cells, leading to unchecked growth that leads to tumors and metastases that can invade the surrounding tissues. It can develop in any tissue or organ and can be diagnosed at any age. However, specific organs, such as the lungs, breasts, prostate, and colon, are the most frequently affected. In addition, the incidence of cancer increases with age, making it the second leading cause of death in industrialized countries, just after cardiovascular disease. Hence, there is an urgency to develop new therapeutic approaches.

Carcinogenesis or oncogenesis, which is the process of cancer formation, is related to the accumulation of direct mutations with age, the presence of viruses or bacteria that hijack cellular functions, or chemical or physical carcinogens that alter the DNA of essential genes such as oncogenes, tumor suppressors, repair and apoptosis genes. However, it is also caused by epigenetic modifications, and histone acetylation and DNA methylation play crucial roles. Dysregulation in these cellular regulatory mechanisms can lead to errors, leading to the expression or overexpression of specific genes such as oncogenes or the inactivation of genes that should be expressed as tumor suppressors, thus disrupting the normal functioning of the cell and creating the loss of cellular homeostasis.

Our understanding of the mechanisms of carcinogenesis has advanced significantly through research in cellular and molecular biology.

In this course on oncology, we will first define several key concepts before further exploring the process of carcinogenesis, such as cell cycle, apoptosis, and telomeres. We will start with an in-depth exploration of the cell cycle at the molecular level. We will examine the cycle’s different phases, stakeholders, regulatory mechanisms that ensure its smooth running, and the main deregulations that can occur, leading to pathological consequences. Next, we will define cell death, shedding Light on the types of cell death, such as apoptosis and necrosis, and discussing the different stakeholders, including the regulatory proteins and signaling pathways involved in these processes.

We will then discuss carcinogenesis, describing the key steps that lead to tumor formation. We will detail crucial cellular and molecular events, such as tumor initiation, promotion, and progression, as well as the metastasis process. We will highlight the genetic and environmental factors that contribute to these processes and expand with concrete examples of the molecular mechanism of cancers.

Finally, we will conclude our study with an overview of cancer therapies. We will mention the most common therapies, such as surgery, radiotherapy, and chemotherapy, and newer treatments, such as immunotherapy and targeted therapy.

1 THE MOLECULAR BASIS OF ONCOLOGY

1.1 The Cell Cycle

The term “cycle" comes from the Greek “kyklos”, meaning “circle”. A cycle refers to an event that repeats itself. The cell cycle is considered a succession of interphase and mitotic division, which is highly regulated, allowing the production of two identical daughter cells from a parent cell. The length of the cell cycle varies depending on the type of cell. It consists of two main phases: the interphase, which is subdivided into three stages: the G1 phase (growth phase preparatory to DNA replication), the S phase (phase of DNA synthesis and duplication), and the G2 phase (second growth phase preparatory to mitosis). Mitosis M is composed of prophase (condensation of chromosomes), metaphase (alignment of chromosomes), anaphase (separation of chromatids), telophase (formation of the two nuclei) and itself includes karyokinesis, followed by cytodieresis (figure:).

Cell division is a fundamental process in eukaryotes, as it is essential for the growth and regeneration of any organism. Cell division plays a crucial role in embryonic development and remains vital throughout the life of the adult organism. Every day, one billion cells need to be renewed to replace those that are continually lost, particularly in the skin, digestive tract, and hematopoietic system. This mechanism of cell division is highly complex. It is regulated by many proteins that intervene transiently and in a precise order, thus guaranteeing the correct succession of the different stages of the cell cycle.

Most cells in an adult organism are not dividing; they are in quiescence, also known as the G0 phase (GAP0). These cells will remain in G0 until they receive a division signal (by mitogenic factors like growth factors that initiate the cell cycle by binding to their receptor), triggering a cascade of intracellular signals that activate signaling pathways, such as the Mitogen-Activated Protein Kinase (MAPK) pathway. This activation leads to the expression of key genes involved in cell cycle progression, including those encoding cyclins and cyclin-dependent kinases (Cdk). This will be followed by the entry into the G1 phase and, therefore, into the division.

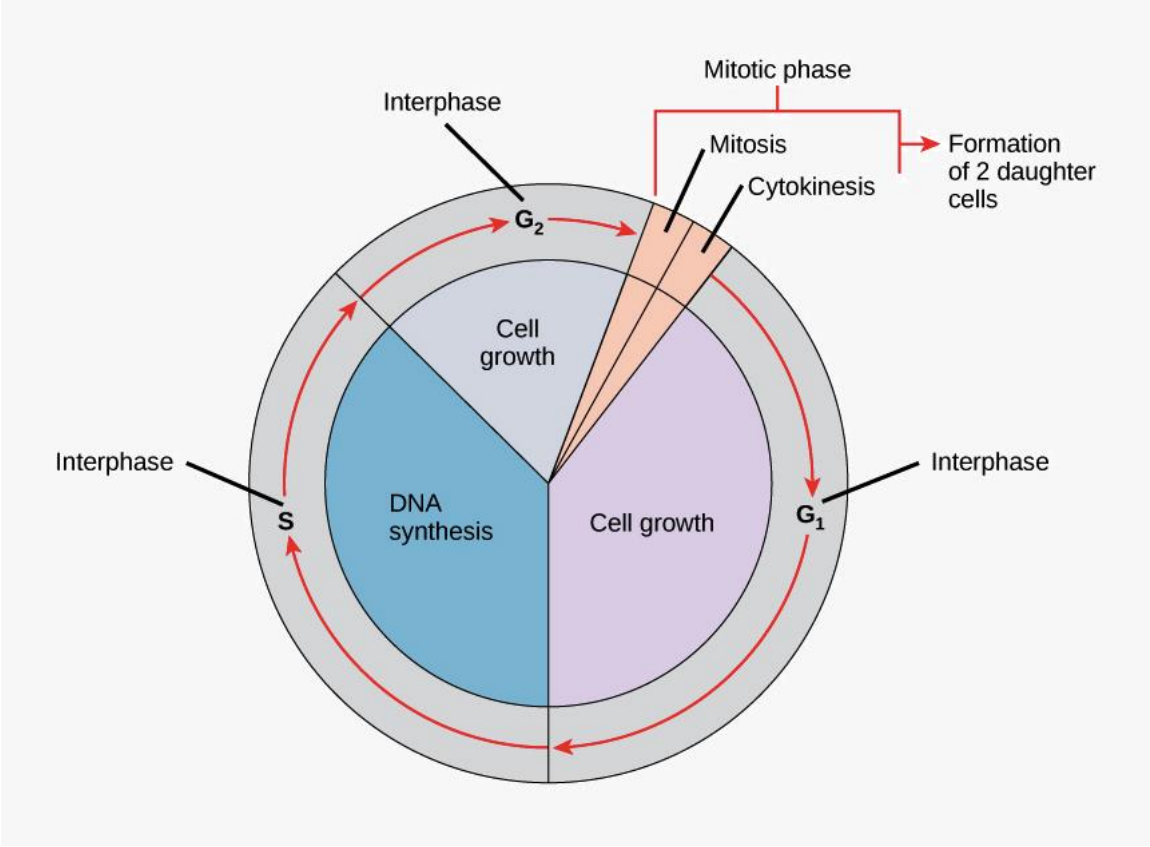


Figure 1: The cell cycle

The relative amount of DNA in a cell at various stages of the cycle of the cell cycle is shown in figure.

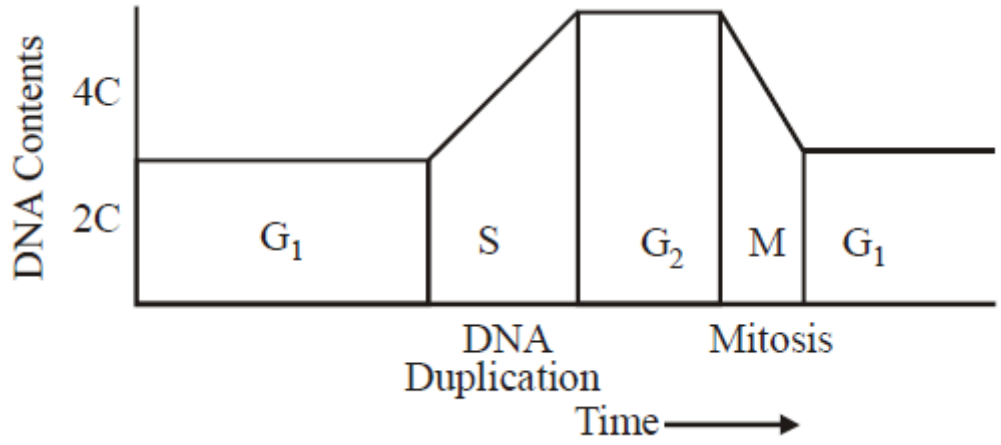


Figure 2: cell cycle stage

The molecular components of the cell cycle and its checkpoints reveal a particular importance in oncology, as dysfunctions in these mechanisms can lead to uncontrolled cell divisions, characteristic of cancers.

1.2 Cell cycle regulation

Cell division is one of the most important biological processes. Depending on the cell type, it may be called mitosis for somatic cells or meiosis for gametes. Given the theme, we will only be interested in mitosis in this course. Mitosis is when a parent cell divides into two daughter cells. It is involved in cell growth, tissue repair, and maintaining cell homeostasis. Mitosis is divided into four major phases: prophase, metaphase, anaphase, and telophase. Careful regulation of the cell division program is crucial for proper cell growth, development, and gametogenesis. Dysfunction in the regulation of cell division can lead to growth defects and proliferative diseases like cancer and age-related diseases, including Alzheimer's disease. Our current understanding of the key regulators of cell division is based on many classic genetic and biochemical studies aimed at understanding the cell cycle. We will start by highlighting the different phases of the cell cycle and the primary regulators. We will try to dissect the molecular mechanisms of cell division and the checkpoints and conclude by using these findings in therapeutics.

2 CELL CYCLE CONTROL MECHANISMS

2.1 Introduction

The cell cycle is a unidirectional and highly regulated process. Interphase, long considered a simple resting phase, is now recognized as a crucial step in regulating the cell cycle.

Two major families of proteins mainly regulate the cell cycle: cyclin-dependent kinases (Cdk) and cyclins. The latter combine to form cyclin/cdk complexes; At least six different complexes are involved at specific times in the control of the cycle.

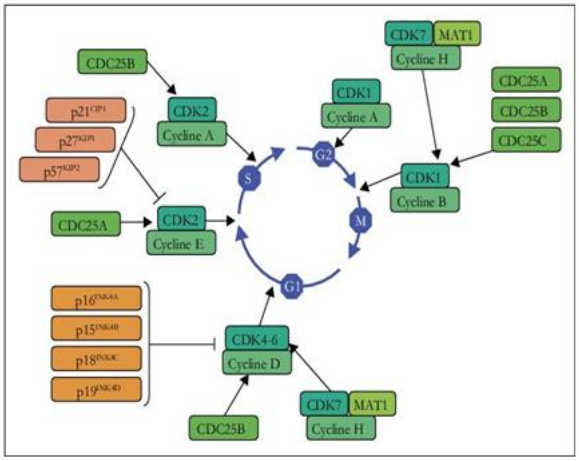


Figure 3 : Schematic representation of the cell cycle stage

2.2 Cyclins

It is a family of several proteins that regulate the cell cycle and is devoid of enzymatic activity. They control the progression of the phases of the cycle by associating with a family of kinases, the Cdk—at least 15 cyclins (A to T) whose expression varies during the cell cycle. Cyclins are absent during the entire cycle. They appear and disappear abruptly at specific times.

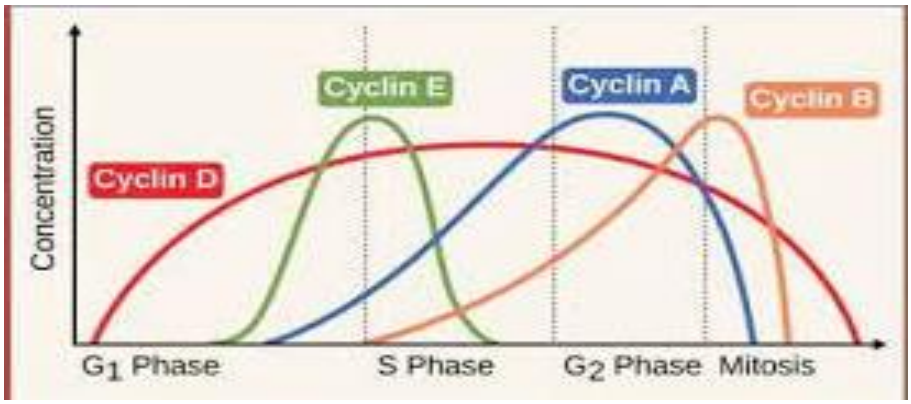


Figure 4 : Fluctuation of Cyclin concentration during cell cycle

2.3 CDKs (or cyclin-dependent kinase)

Cyclin-dependent kinases (Cdk) play a critical role in the regulation of the cell cycle of eukaryotes. Their activation is chronologically regulated by post-translational modifications, such as phosphorylations and dephosphorylations, and by their association with cyclins, which act as regulatory subunits of the enzyme complex. These serine-threonine kinases catalyze the phosphorylation of various target proteins involved in cell cycle events (DNA replication, nuclear envelope fragmentation, DNA condensation, etc.). Cdks regulate the cell cycle by, for example, phosphorylating the Rb protein, interacting with transcription factors, and phosphorylating histone H1 and other components of the mitotic apparatus. Deregulation of Cdk can contribute to the development of neoplastic processes.

Cyclins are absent without stimulation, thus rendering the Cdks free and inactive. Cdks comprise two lobes: an N-terminal lobe rich in beta sheets and a C-terminal lobe rich in alpha helices. The intersection of these two domains forms a pocket corresponding to the catalytic site where ATP binds. Before stimulation, the catalytic site of the kinase is inaccessible to ATP due to a blocked configuration. The PSTAIRE (named after the amino acids of which they are composed) and T-loop (which contains CAK-phosphorylated threonine-160) loop mask the entrance to the catalytic site, thus constituting Cdk regulation zones (figure).

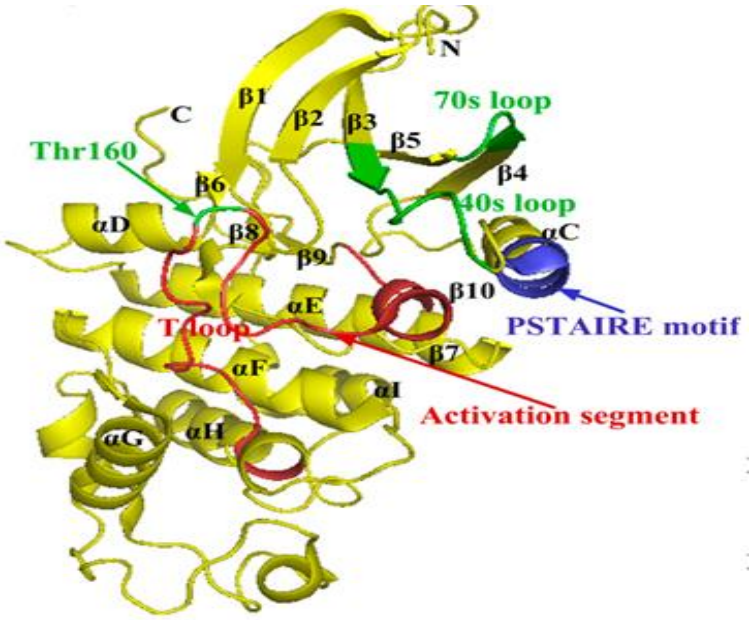


Figure 5: Structural representation of monomeric Cdk2

Cyclin interacts with the terminal N and C lobes, directly contacting the PSTAIRE and T-loop domains. These interactions result in conformational changes: the T-loop (activation domain) is displaced by 20 angstroms, while the PSTAIRE (in helix) propeller undergoes a 90-degree rotation (Figure 6). This helix movement provides an optimal structure that allows the fixation and orientation of ATP for the phosphate transfer reaction to the substrate. The movement of the T-loop provides access to the catalytic site, which is typically obstructed.

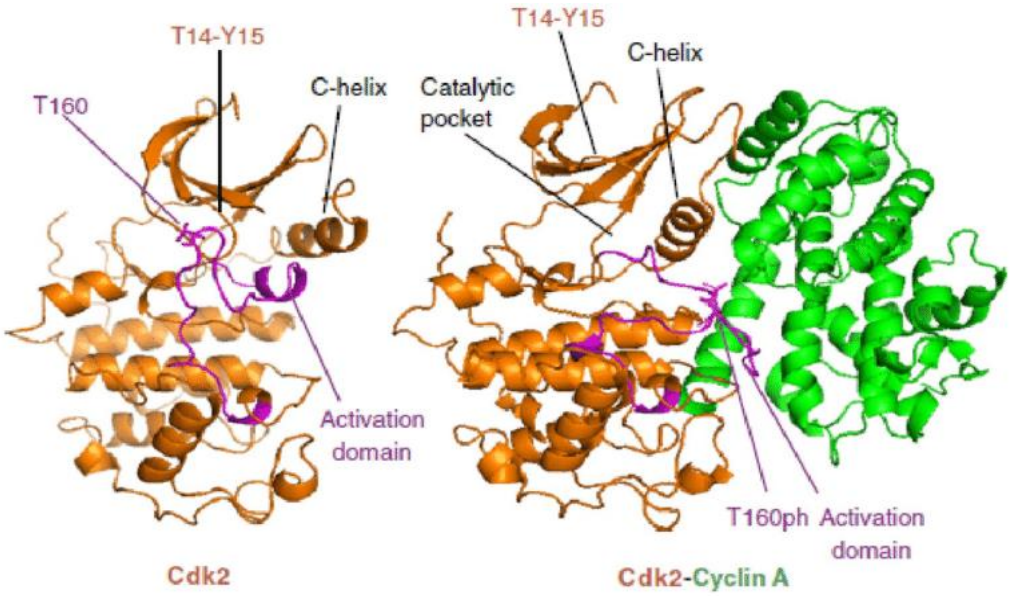


Figure 6 : comparaion of Structural representation of A)cdk2 monomeric B) Cdk2/Cyclin A complex

2.4 Complex control

2.4.1 Establishment of a complex pre-stock, e.g., inactive cyclin B/Cdk1 (Pre-MPF)

The MPF (mitosis-promoting factor) is none other than the Cyclin B / Cdk1 complex. It is one of the most studied complexes of the cycle, given its importance because it represents the second checkpoint of the G2 stage and allows the transition to M after ensuring the proper functioning of S. Cyclin B accumulates gradually during the S and G2 phases, resulting in a gradual increase in the Cyclin B/Cdk1 complex as the cell approaches mitosis. Cdk1 is phosphorylated on threonine-161 (at the T-loop) by CAK (CDK activating kinase) (Cyclin H / Cdk7) to stabilize this structure and maintain the openness of the site (active phosphorylation). However, this complex remains inactive due to the phosphorylation of two neighboring amino acids, tyrosine 15 and threonine 14, by the Wee-1 (Myt-1) protein of the PSTAIRE loop, thus preventing the entry of the substrate (inactivating phosphorylation). Thus, at the end of the G2 phase, the cell has an abundant stock of Cyclin B/Cdk1 (inactive pre-MPF) ready to act but whose activity is repressed by the presence of two phosphate groups that block kinase activity. Pre-MPF = Cyclin B/Cdk1 (Tr 161p, Tr 14p, Ty 15p).

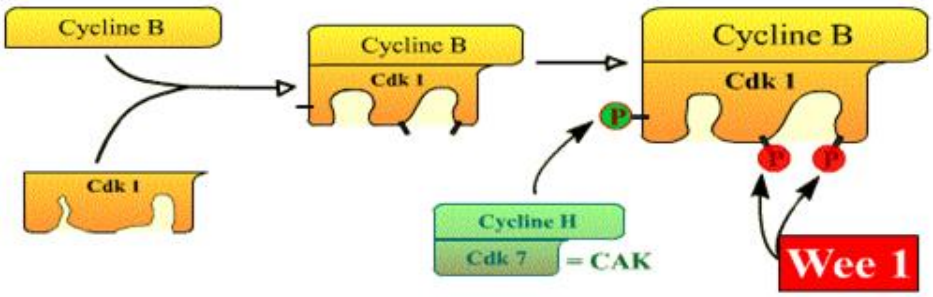


Figure 7 : Regulation of Pre-MPF

2.4.2 Rapid activation of Cyclin B/Cdk1

Cyclin B/Cdk1 activation results from a series of interrelated reactions. Once replication is complete, Cdc25 phosphatase is no longer retained in the cytoplasm and migrates to the nucleus. At the end of the G2 phase, Cdc25 is activated, probably by phosphorylation by the Polo kinase. This activation allows Cdc25 to remove both inhibitory phosphates from Cyclin B/Cdk1, activating a small fraction of this complex.

Cdk1 can then phosphorylate Cdc25 at a site different from the Polo kinase's. This phosphorylation activates Cdc25, thus allowing Cyclin B/Cdk1 to activate its activator, illustrating a self-activation process. At the same time, Cyclin B/Cdk1 phosphorylates Wee1, thereby inhibiting this protein, which means that Cyclin B/Cdk1 also inhibits its inhibitor.

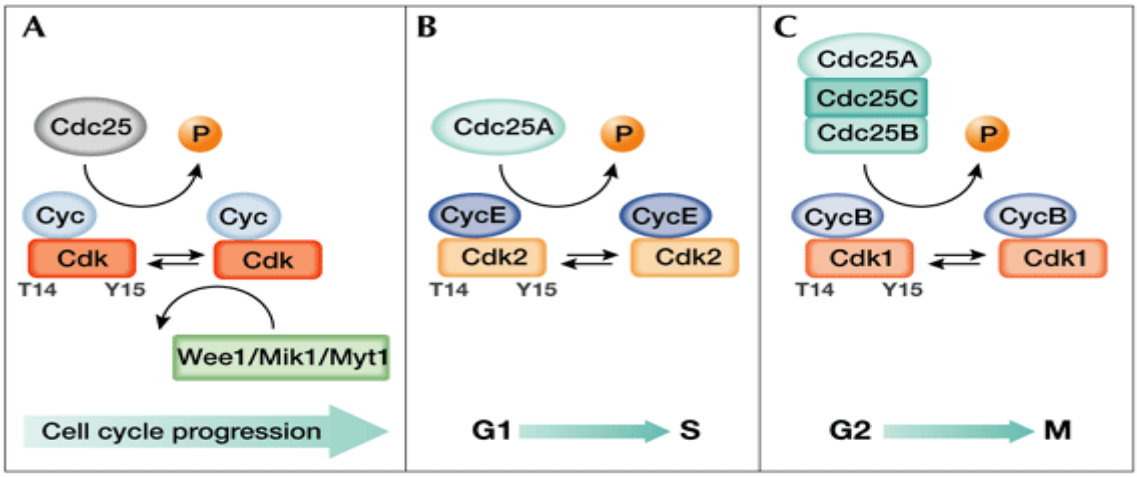


Figure 8 : Activation of Cdk/cycline complex

This dual positive feedback mechanism allows many active Cyclin B/Cdk1 complexes to be obtained quickly and irreversibly, thus illustrating positive feedback.

2.5 Cell cycle inhibitors (CKIs)

CDKs are so essential that they cannot be controlled solely by association with cyclins, phosphorylation, and dephosphorylation cycles. A family of proteins known as CKIs (CDK inhibitors) also plays a crucial role in regulating CDKs.

2.5.1 Regulation of Cyclin/CDK complexes by CDK Inhibitors

One of the key mechanisms regulating the activity of Cyclin/CDK complexes is their interaction with inhibitory proteins. A total of seven CDK inhibitors, the CKIs, have been identified and are divided into two families: the Ink4 family specific to the CDK4 and CDK6 kinases (p16, p15, p18, and p19) and the Cip/Kip family binds to Cyclin/CDK complexes by forming heterotrimers. (p21, p27 and p57).

p21 is one of the primary mediators of cell cycle arrest in response to various stresses, its transcription being able to be activated by these and dependent on p53, which acts as a

transcription factor. Thus, different disturbances (such as DNA damage, hypoxia, or overexpression of oncogenes) increase p53 levels.

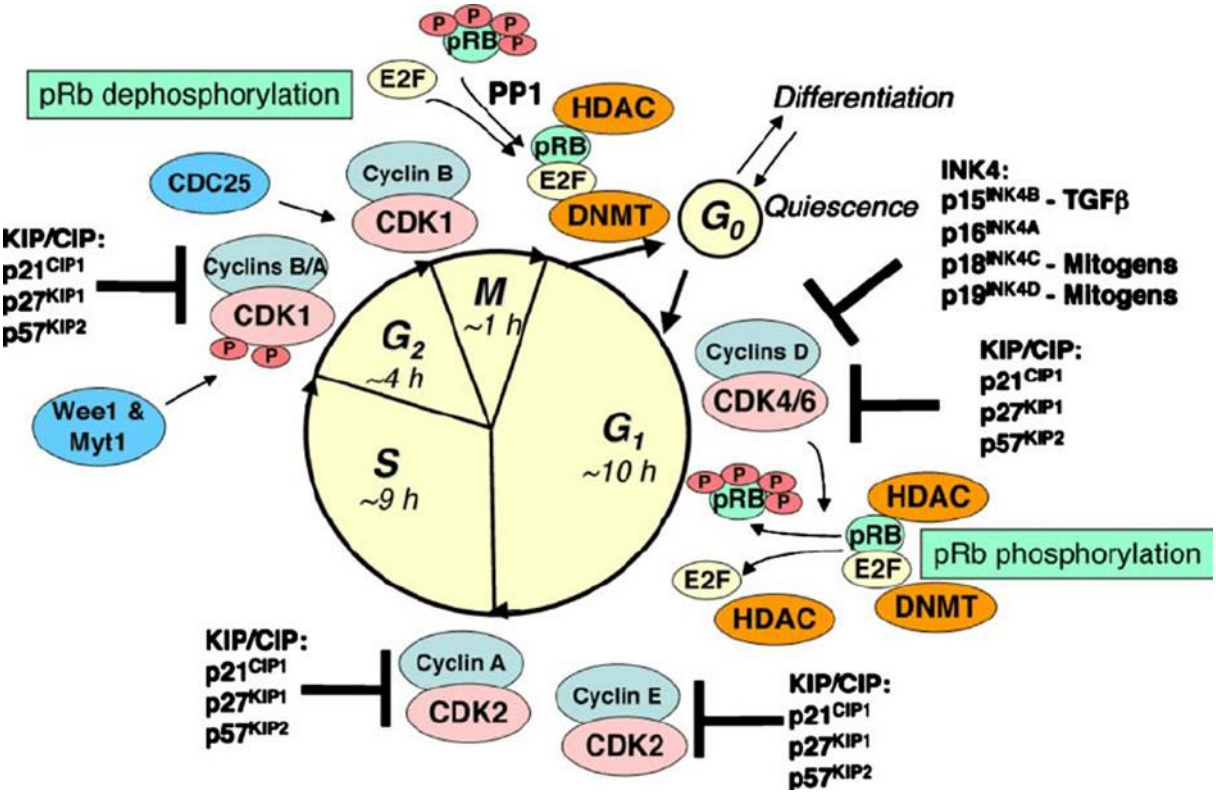


Figure 9: Cell cycle inhibitors

2.6 Cell cycle checkpoint regulation

The cell cycle is controlled at three points of restriction, between G1 and S, G2 and M, and M. Checkpoints in the cell cycle play a crucial role in ensuring that each step takes place correctly, thus maintaining the integrity of the genetic material and preventing errors that can lead to diseases such as cancer.

2.6.1 G1-S Control Point:

The G1/S checkpoint is the first checkpoint of the cell cycle. It is located between the G1 phase and the S phase. It regulates the commitment of eukaryotic cells to enter the DNA synthesis (S) phase, where DNA is replicated after checking if the environmental conditions are favorable (cell size, nutrient availability...) and the genetic material is intact.

2.6.1.1 The pRb protein

The Rb gene (retinoblastoma, a retina cancer), is located on chromosome 13q14. It encodes a 928-amino acid phosphoprotein (105 to 110 kDa) that regulates cell proliferation, particularly the entry into the S-phase of the cell cycle. Located in the nucleus, the pRb protein acts as a negative regulator of the cell cycle, playing an essential role in cancer prevention. Mutations in the Rb gene can disrupt this control, thus contributing to oncogenesis, particularly at the origin of retinoblastoma.

The pRb protein has several domains (Figure): a protein-binding domain (A/B pocket, which interacts with the transcription factor E2F), a DNA-binding domain, and various motifs allowing interactions with other regulatory proteins. Its structure allows it to modulate transcriptional activity in response to intracellular signals. In humans, pRB has three domains:

- pRB_A: Cyclin binding site,
- pRB_B: Domain of linking. Site of interaction with viral proteins,
- pRB_C: Carboxyl-terminal domain. A binding site is required to bind the E2F protein. Phosphorylation site of CDK4 and CDK

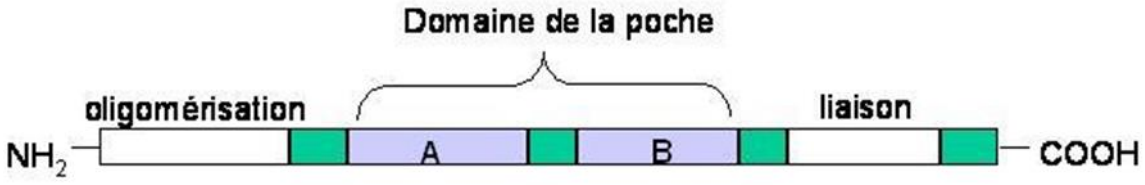


Figure 10 : structure of the pRb protein

2.6.1.2 Role of the pRb protein:

In cells not engaged in G1, pRb is hypo-phosphorylated and binds to transcription factors such as E2F, thus inhibiting essential events. pRb is phosphorylated by the cyclin complexes D: Cdk4/6 and cyclin E: Cdk2. These two kinase complexes are required to phosphorylate pRb entirely and block the interaction between pRb and E2F, which allows the transcription of genes necessary for DNA replication. Various stimuli, such as TGF-β and DNA damage, influence this checkpoint by activating CKIs such as P21 by activating P53, causing the cycle to stop. TGF-β also inhibits the transcription of cdc25A, a phosphatase required for CDK activation.

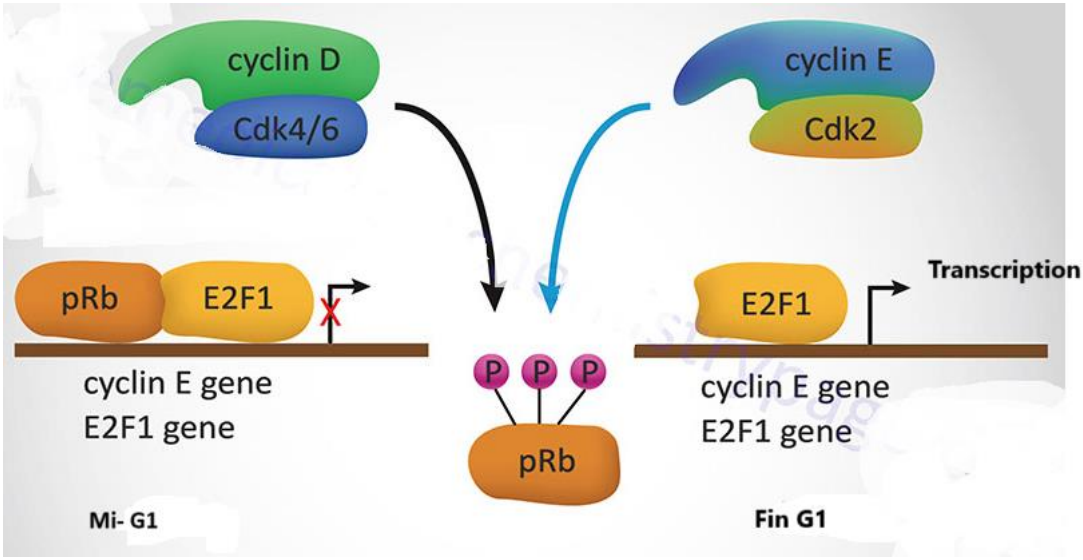


Figure 11: Mecanism of regulation of E2F by pRb

E2F-dependent genes encode regulatory proteins such as cyclin E and Myc protein and genes whose products are enzymes involved in replication that enable S entry. These genes are activated by E2F1:DP1 complexes (and other E2F heterodimers) and negatively regulated when E2F is bound to pRb.

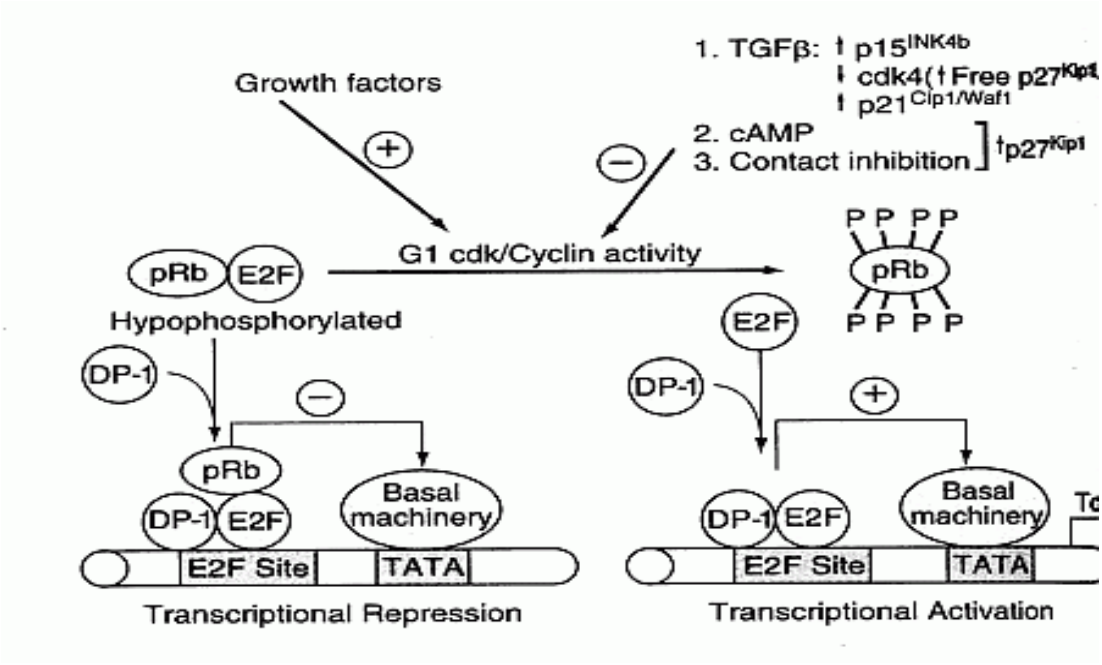


Figure 12: Regulation of Transcriptional Activity by pRb and E2F in Cell Cycle Control

The pRb (retinoblastoma protein) is key in controlling this checkpoint. If DNA damage is detected by surveillance proteins such as ATM and ATR, it activates kinases like Chk1 and Chk2, which phosphorylate and inactivate CDKs, blocking progression. In the event of DNA

damage or other stress signals, the p53 protein can also be activated, activating p21cip1, a cyclin/CDK inhibitor, thereby blocking the G1-S transition.

2.6.1.3 Rb gene dysfunction

Mutations affecting the biological function of pRB are usually single-base-pair point mutations, leading to nonsense mutations. These mutations prevent the protein from properly sequestering the E2F/DP complex. If this complex is not properly sequestered, the transcription factor E2F can cause the cell cycle to continue. Since pRB is a monomeric protein, it is necessary to have the two alleles of the mutated RB1 gene to manifest the pathological condition.

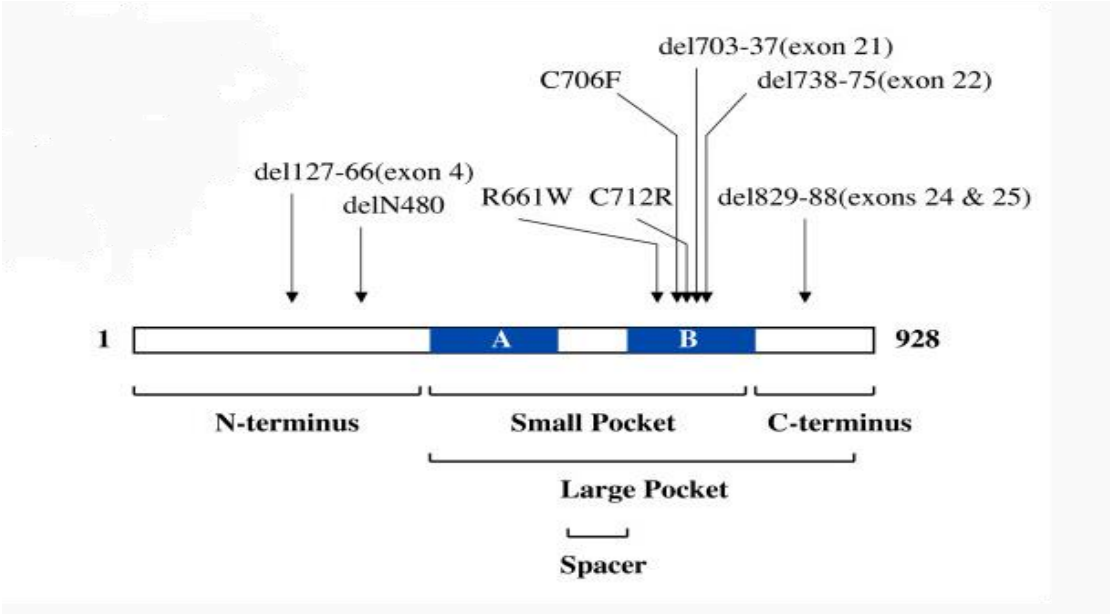


Figure 13 : Characterization of pRb Protein and Associated Mutations in the Pocket Domain

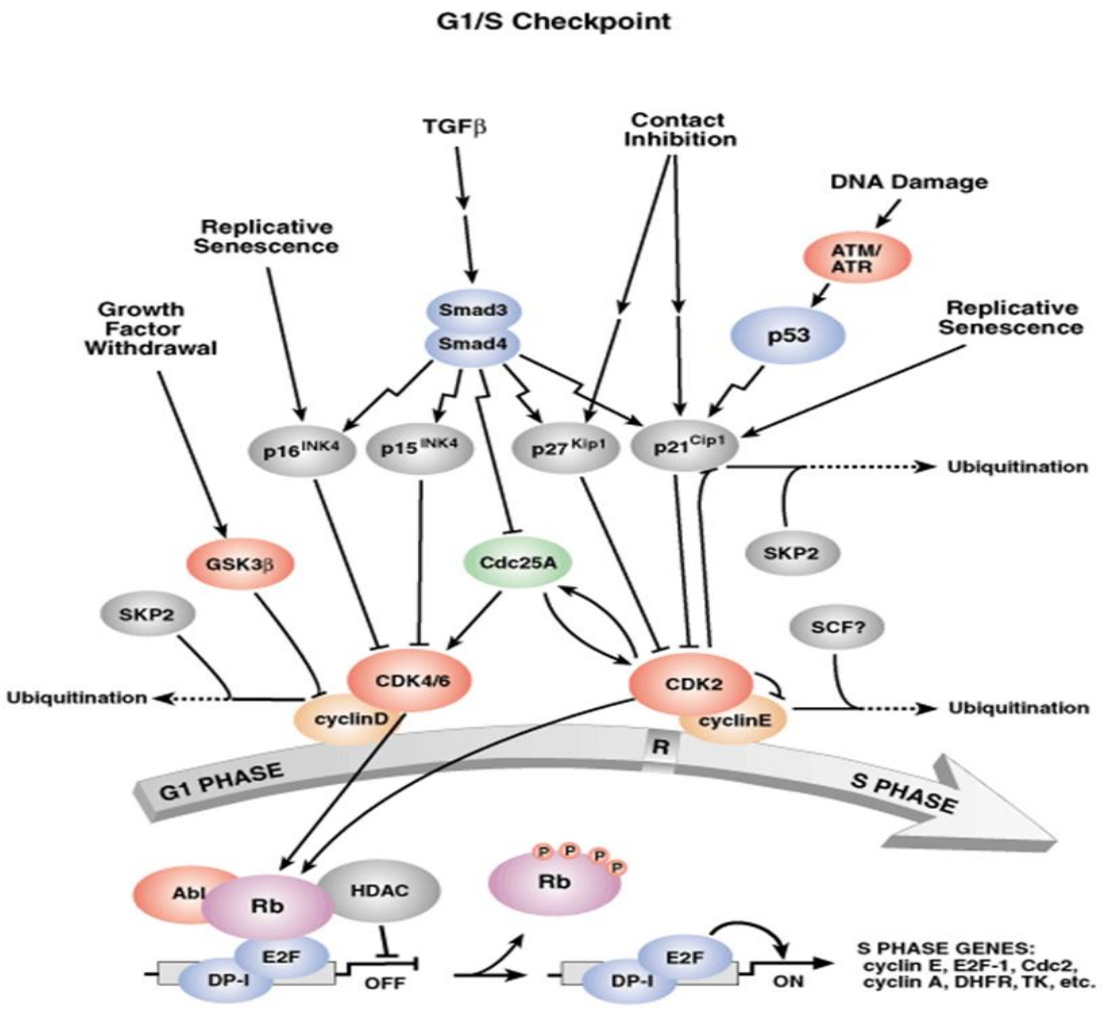


Figure 14 : G1-S checkpoint regulation

2.6.2 G2-M checkpoint

This checkpoint ensures that the DNA has been completely and correctly replicated before granting permission to the cell to enter phase M. The MPF (mitosis-promoting factor) plays a key role in this control.

The transition from the G2 phase to mitosis (M) is mainly regulated by the cyclin B/CDK1 complex, also known as the maturation promoter factor (MPF).

Phosphorylation of Cdc25C on serine-216 promotes its interaction with the protein 14-3-3σ, which inhibits its phosphatase activity. In addition, the binding of 14-3-3σ also stimulates the activity of Wee1, adding downregulation on G2→M progression. Chk1, Chk2, or Plk3 can phosphorylate Cdc25C. ATR mainly activates Chk1, while ATM or ATR phosphorylates Chk2. ATR is activated in response to replication problems caused by damage such as ultraviolet

radiation. In contrast, ATM is activated by double-strand breaks in DNA, including those induced by ionizing radiation.

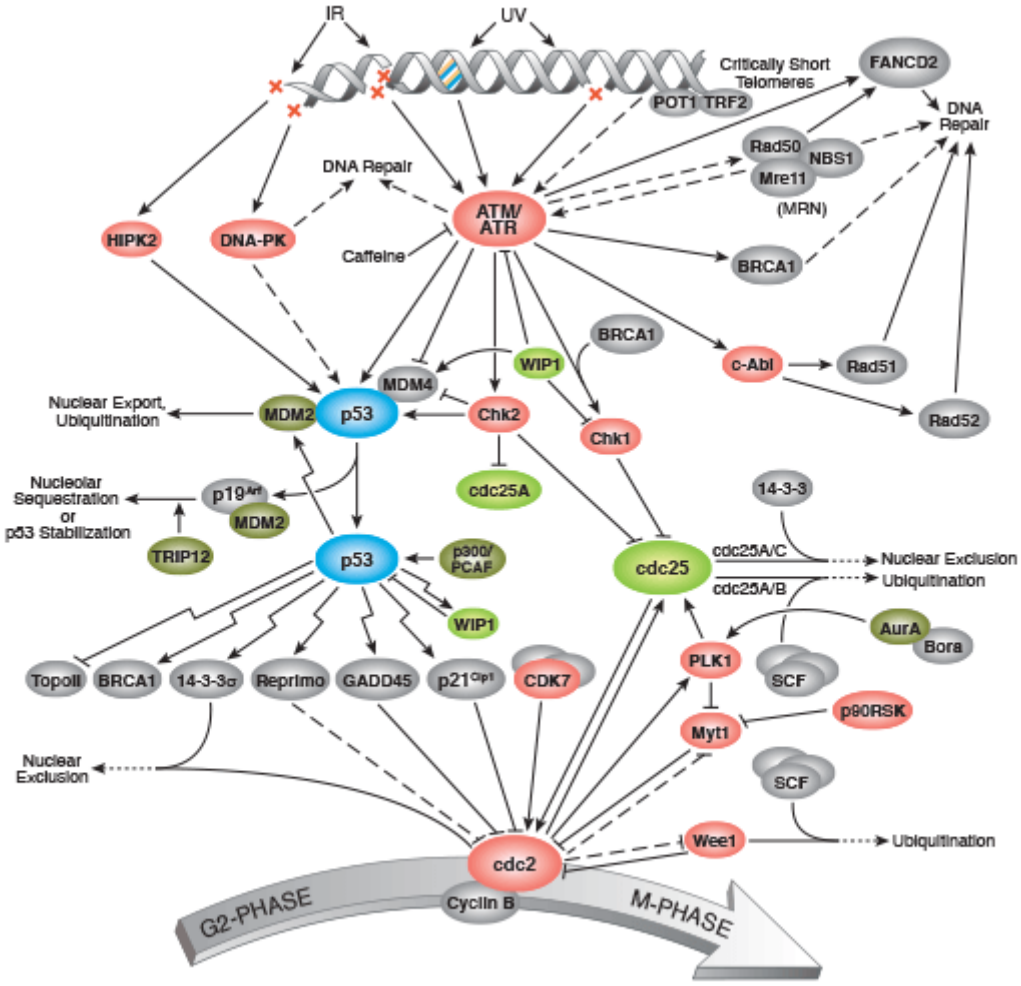


Figure 15: G2-M checkpoint regulation

ATM can also phosphorylate p53 on serine-15, thus stabilizing its protein by limiting its degradation. Chk1 and Chk2 phosphorylate p53 on serine-20, which prevents the binding of Mdm2, a protein that generally results in the degradation of p53 by ubiquitinylation. Mdm2 also binds to p14Arf, which decreases the degradation of p53. In addition, Mdm2 interacts with E2F1 to stimulate its degradation, thus blocking the activation of genes involved in the S phase. The p53 protein, on the other hand, activates several genes, including those encoding Mdm2, p21cip1, Gadd45, 14-3-3, and Bax, while inhibiting genes such as cyclin B1 and CDK1. Finally, Chk1 is activated by UV and replication anomalies, while Chk2 responds to ionizing radiation. Plk3, activated by ATM and ATR, phosphorylates both p53 and Cdc25C and is activated by agents such as UV and oxidative stress.

3 THE GUARDIAN OF THE GENOME: THE P-53 PROTEIN

The p53 protein is the guardian of the integrity of the cell's genetic material. It is found to be expressed in cells subjected to stress and, in particular, during stress-inducing mutations. Its involvement in almost all the stages leading to tumor development makes its study particularly interesting. Like pRb, p53 is a tumor suppressor.

3.1 Structural and Functional Aspect of the p53 Protein

The human p53 protein is a phosphoprotein of 393 amino acids, organized into several functional domains characteristic of a transcription factor. Here is an overview of its main areas:

- **N-terminal domain (residues 1-42):** Essential for interaction with components of the transcriptional apparatus and transactivation of target gene expression.
- **Proline-rich region (residues 63-97):** Involved in apoptotic processes.
- **Core domain (residues 100-300):** Contains the DNA-specific binding domain (DBD). This structured, evolutionarily conserved region binds to ER (p53 response elements) present in p53 target genes, thus stimulating their expression.
- **Tetramerization domain (residues 335-356):** Facilitates the formation of p53 tetramers, which is essential for its action.
- **C-terminal domain (residues 363-393**): Involved in downregulating p53 activity and interacting with single-stranded DNA.

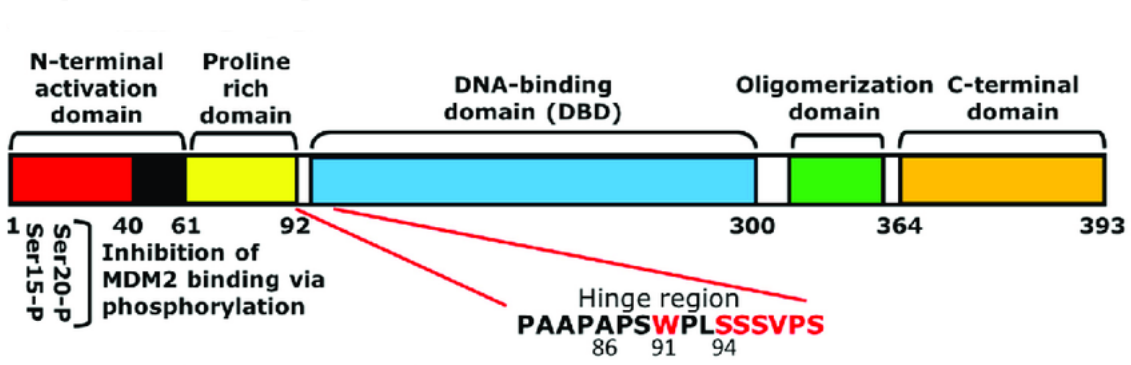


Figure 16 : Schematic representation of P53 domain

3.2 Location and Structure of the p53 Gene

The gene encodes the p53 protein on the short arm of chromosome 17, precisely in the 17p13.1 band. This gene consists of 11 exons and spans a total length of 20 kilobases (kb). An intron sequence of 10 kb is found between the first and second exons, while the highly conserved regions during evolution are located mainly between the second and eighth exons. The central domain of p53 is usually encoded by exons 5 to 8.

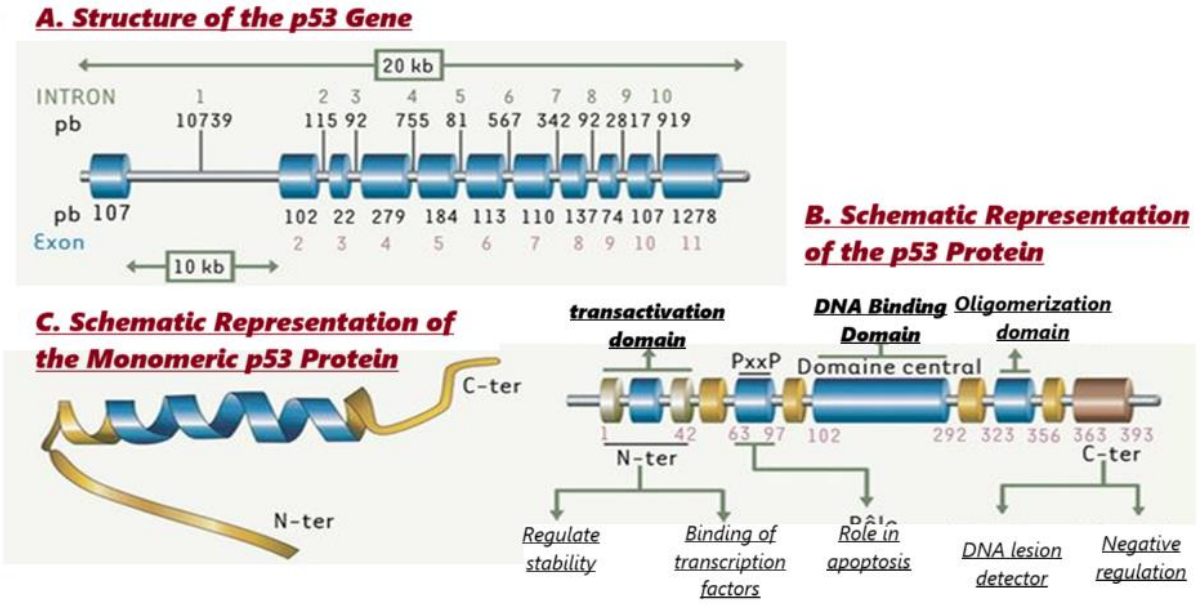


Figure 17 : Schematic representation of P53, A) gene structural representation, B) schematic representation of p53 protein, C) schematic Representation of the p53 Protein Monomer

3.3 p53 Function & Regulation

Under physiological conditions, the p53 protein is present at low levels in the cell. During its synthesis in the cytoplasm, it is rapidly bound by the Mdm2 protein, an E3 ligase, which adds ubiquitin chains on p53, resulting in its degradation by the proteasome. In cellular stress, such as irradiation, the interaction between p53 and Mdm2 is abolished, allowing p53 to migrate to the nucleus. Under these conditions, the transcription factor E2F1 is strongly expressed, generating the expression of the ARF protein. The latter can degrade Mdm2, thus stabilizing p53 and increasing its intracellular level. This is the first phase of the activation phase.

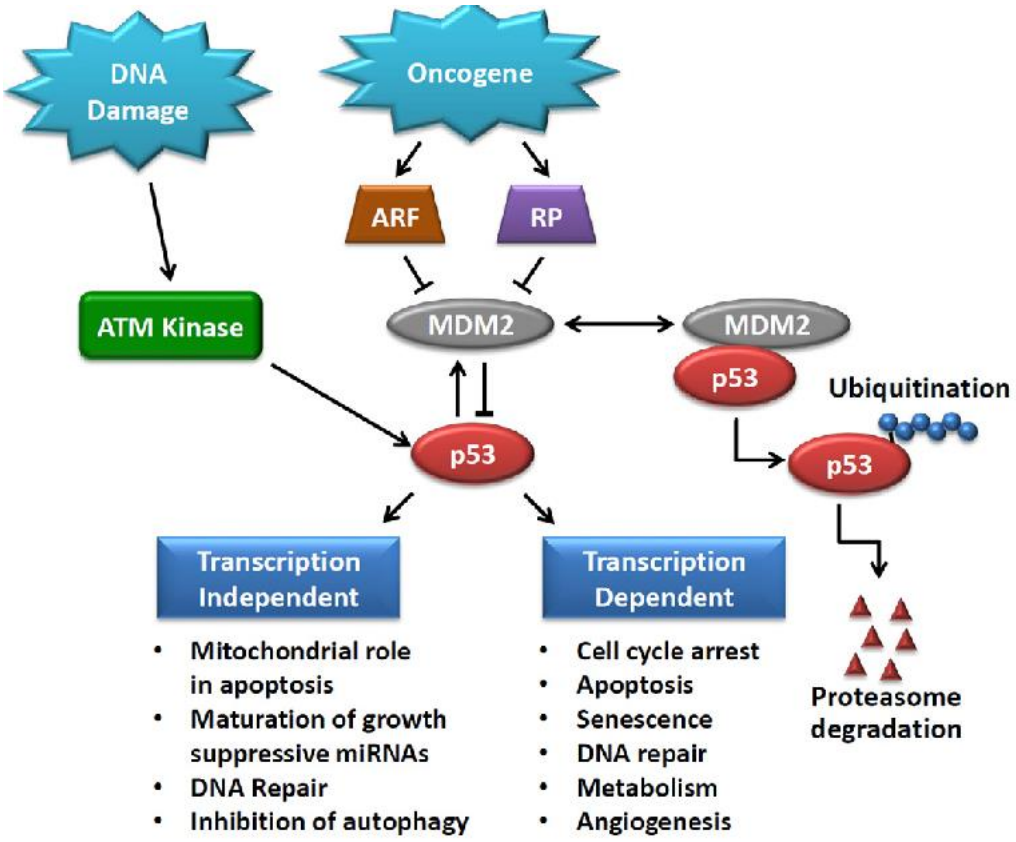


Figure 18: schematic representation of p53 regulation

In the second step, p53 will undergo several post-translational modifications of its different regions of different types: acetylation, phosphorylation, Adenylation... etc. (figure), which will allow its activation as a transcription factor and its regulation, this is the modification phase.

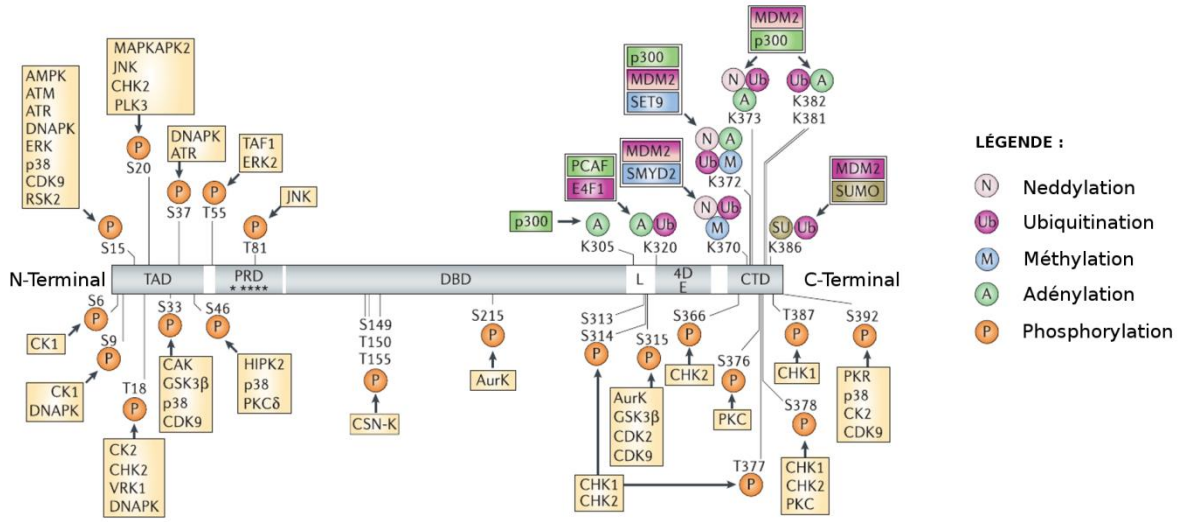


Figure 19: post-translational modifications and regulation of p53

It should be noted that the detailed post-translational changes here are far from the only ones; p53 is an extremely regulated protein, and its post-translational modifications are very complex.

Thirdly, like any transcription factor, P53 can bind to DNA to activate the genes it activates or represses, which is the response phase.

The selection of target genes by p53 is a complex process. It depends on several factors, including the affinity of p53 for the different promoters, the associated cofactors, and its post-translational modifications. The target genes of p53 are numerous and diverse, varying according to various stimuli (figure). The target genes of p53 can be classified into five major groups corresponding to the other cellular responses: cell cycle arrest, senescence, apoptosis, DNA repair, and differentiation. We can also mention Mdm2, a target gene of p53, thus allowing negative feedback. When the cell has successfully repaired the damage, the increase in Mdm2 leads to a degradation of p53, facilitating the resumption of the cell cycle.

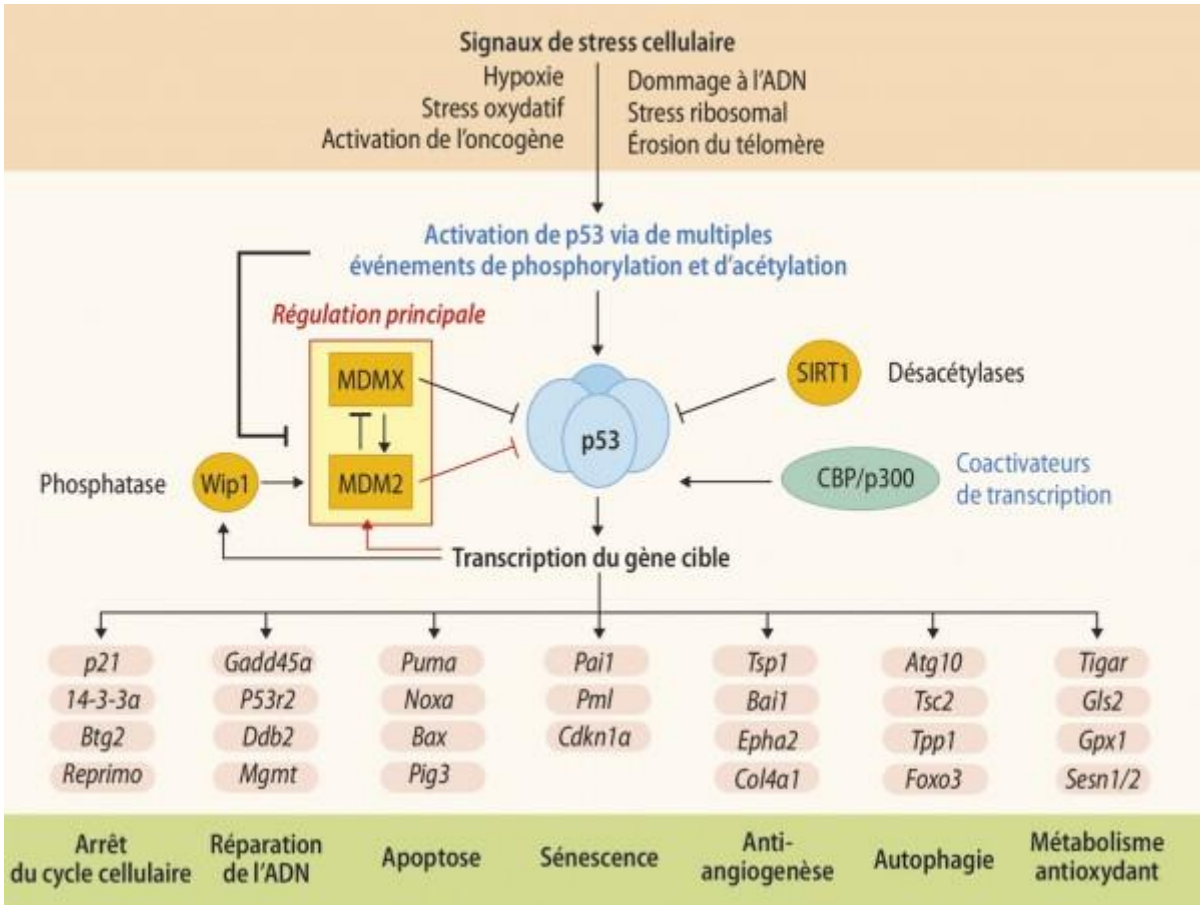


Figure 20 : Cell Stress Signals and the Regulatory Network of p53 Activation

3.4 Inactivationμ of p53 in cancers:

The loss of p53 requires the inactivation of both alleles of the p53 gene. The p53 protein is one of the most studied transcription factors due to its critical role in cancer, with the TP53 gene mutated in more than half of sporadic cancers and most other cancers with overexpression of Mdm2 or Mdm4, which downregulate p53. Germline mutations in TP53 are also responsible for Li-Fraumeni syndrome, which predisposes to cancer. In response to various cellular stresses such as DNA breaks and oncogenic signals, p53 is stabilized and activated, inducing the transcription of genes involved in cell cycle arrest, senescence, and apoptosis, which contributes to its function as a tumor suppressor and earns it the title of "guardian of the genome". The majority of mutations in TP53 are missense mutations, affecting its DNA-binding domain and reducing its ability to regulate target genes (figure). The inactivation of p53 is mainly due to these missense mutations in about 75% of cases in humans, 95% of which are in the DNA-binding domain, thus preventing p53 from binding to DNA and regulating gene expression in response to cellular stress.

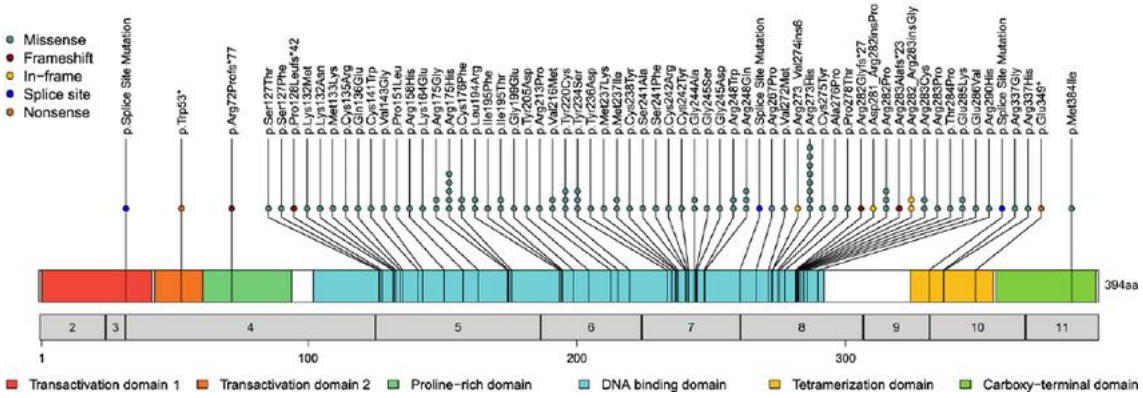


Figure 21 : Location, frequency, and type of TP53 mutations

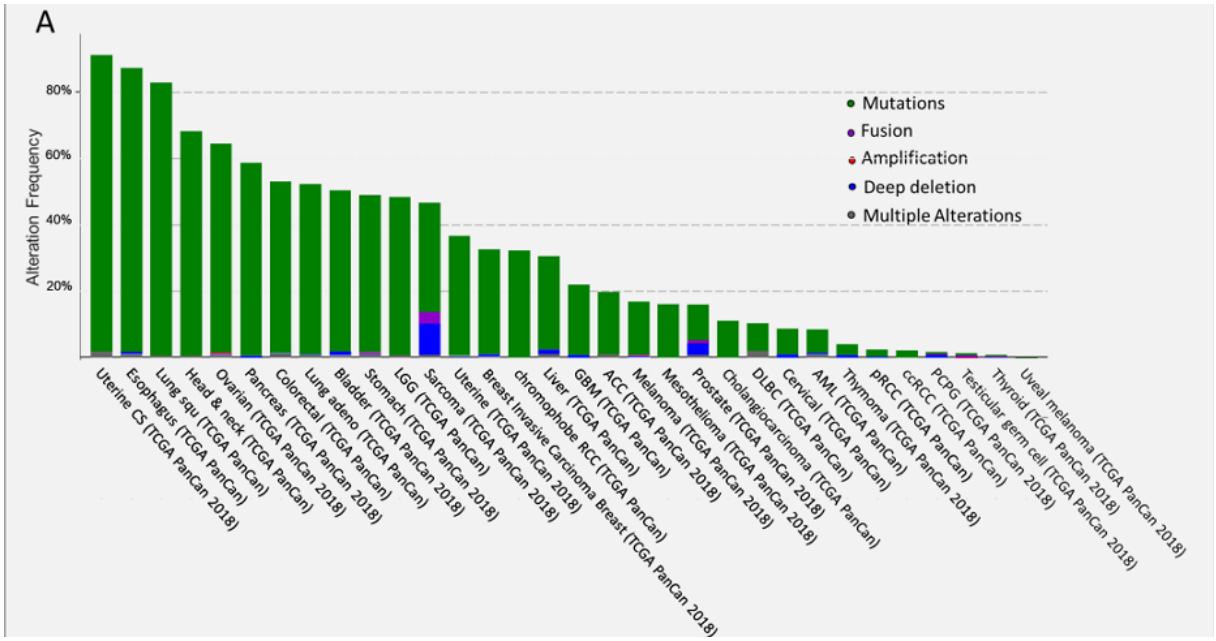


Figure 22: Mutation features of TP53 in tumors

The interaction between apoptosis and the cell cycle is essential for a harmonious regulation of cell proliferation and cancer prevention.

Apoptosis, or programmed cell death, is a biological process crucial for the removal of damaged, unnecessary, or unwanted cells, thus contributing to the maintenance of tissue homeostasis. Its role is particularly important in cancer prevention, as the accumulation of abnormal cells can lead to genetic changes that cause tumors. In carcinogenesis, apoptosis serves as a protective mechanism by controlling cell proliferation, especially in response to abnormalities such as DNA damage or oncogenic signals. This process is closely linked to the cell cycle, which regulates cell division and growth. When apoptotic pathways are altered, as is often the case in cancers, abnormal cells can escape this control and multiply uncontrollably, promoting tumor development. Thus, the study of apoptosis and its integration into the cell cycle is therefore of particular importance, both for the understanding of the mechanisms underlying carcinogenesis and for the development of new therapeutic strategies aimed at restoring the apoptotic process in order to eliminate cancer cells. To do this, we will try to define and explain apoptosis.

4 PROGRAMMED CELL DEATH

4.1 Introduction

There are several forms of cell death, each with distinct characteristics and mechanisms. Three are the most recognized: apoptosis, necrosis, and autophagy (figure). Apoptosis is a programmed, often ordered, cell death involving caspases and not causing inflammation. Necrosis, on the other hand, is accidental and leads to cell rupture and an inflammatory response. Autophagy is a regular and orderly process, somewhat different at first glance because it is a mechanism for the survival of the cell from a nutrient deficiency, the destruction of the cell in this case is done by its own lysosomes forming the autophagosomes. The self-digestion of its organelles allows the cell to meet its needs for substrates. But there are also other deaths such as pyroptosis, which is an inflammatory death linked to infections; ferroptosis, which results from an accumulation of lipid peroxides; necroptosis, which is a programmed form of necrosis, controlled by specific mechanisms; and finally, entosis, which involves the engulfment of one cell by another, which can have implications for tumor progression. Each of these forms plays a key role in regulating cellular health and maintaining tissue homeostasis. Although several types of cell death are implicated in cancer, apoptosis is one of the most important, as its dysregulation contributes significantly to cancer pathology.

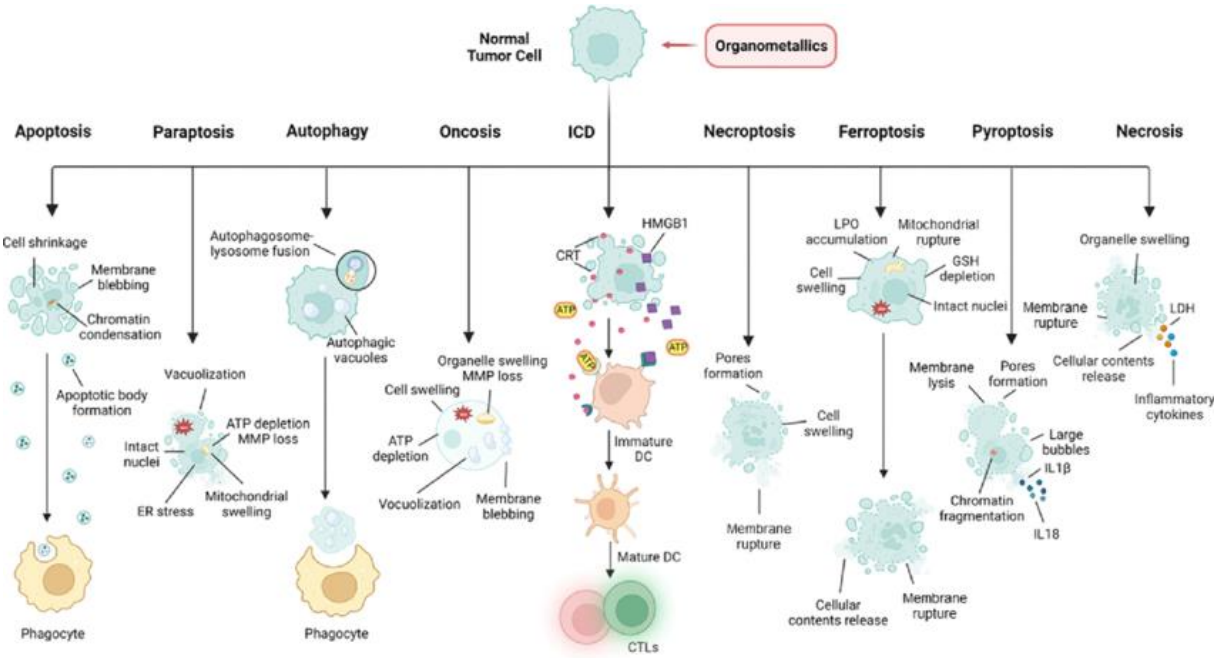


Figure 23: representing the main cell deaths

4.2 The regulation of apoptosis

Cell death, which plays a crucial role in the development and progression of cancers, is called apoptosis. This name refers, etymologically, to the programmed fall of the leaves in autumn; **"apo"** for distance and "**ptosis**" for fall. Apoptosis is a process of programmed cell death that removes damaged or potentially dangerous cells in a controlled manner without causing inflammation. Apoptotic cell death is involved in regulating the immune system, eliminating cells during embryonic and fetal development, and eliminating interdigital tissues.

In many cancers, tumor cells develop mechanisms to evade apoptosis, allowing them to survive and proliferate uncontrollably. Among the disturbances that can affect this process are mutations in apoptosis-regulating genes such as p53, Bcl-2, and others. These alterations can make cancer cells resistant to treatment and improve their longevity, promoting tumor progression. Apoptosis can be triggered by various signals such as DNA damage, cellular stress signals (such as oxidative stress, hypoxia, or lack of nutrients), signals from other cells (cytokines, death ligand Fas or necrosis factor TNF), growth factor deprivation or viral infections. These triggers mainly activate two pathways, the intrinsic pathway or mitochondrial pathway, and the extrinsic pathway, which can be interconnected.

Two major families are involved in this physiological process: the Bcl-2 and caspase families.

4.3 The Bcl-2 family of proteins

The Bcl-2 (B-cell lymphoma 2) family of proteins plays a crucial role in regulating apoptosis. These proteins include pro-apoptotic (Bcl-2, Bcl-XL, and Mcl-1) and anti-apoptotic (Bax, Bad, Bak, and BH3 only Bim, Bid, PUMA regulators) that interact to determine the fate of a cell (figure).

The Bcl-2 protein, composed of 239 amino acids, is encoded by a gene located on chromosome 18 at the q21.33 locus. It has four homology domains, present in other proteins of the same family, as well as a transmembrane domain that gives it its apoptotic activity (figure). Named after B-cell lymphoma, Bcl-2 (B-cell lymphoma2) became famous because of its discovery in connection with this cancer, This cancer is due, in large part, to a translocation of chromosomes 14 and 18. This translocation places the Bcl-2 gene following the promoter of the immunoglobulin heavy chain gene. This has the effect of creating an overexpression of Bcl-2 and, therefore, substantially increasing the cell survival of tissues normally expressing the immunoglobulin gene, hence lymphoma.

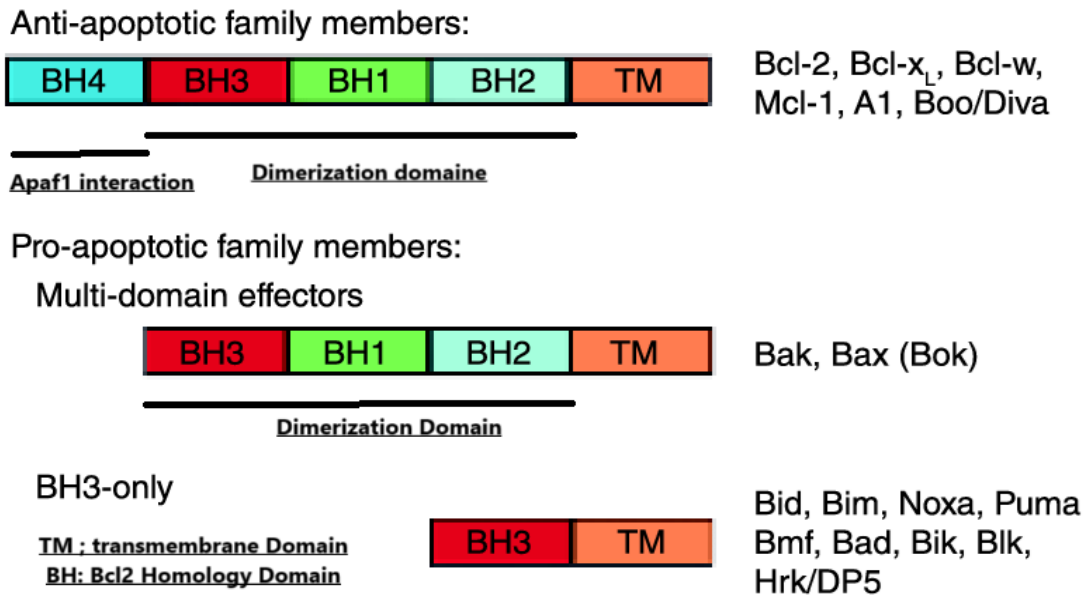


Figure 24: structure of Bcl-2

Localized in the mitochondrial membrane, Bcl-2 inhibits apoptosis by preventing the homodimerization of Bax, Bak, and other pro-apoptotic proteins. In contrast, Bcl-2 can be inhibited by Bad, a BH3-only protein in the Bcl-2 family. Under pro-apoptotic conditions, Bad binds to Bcl-2, preventing Bcl-2 from binding to multi-domain pro-apoptotic proteins such as Bax and Bak. In this context, Bcl-2 can no longer inhibit the homodimerization of Bax and Bak, thus allowing the formation of pores in the mitochondrial membrane, which releases cytochrome c in the cytosol and activates the apoptotic process.

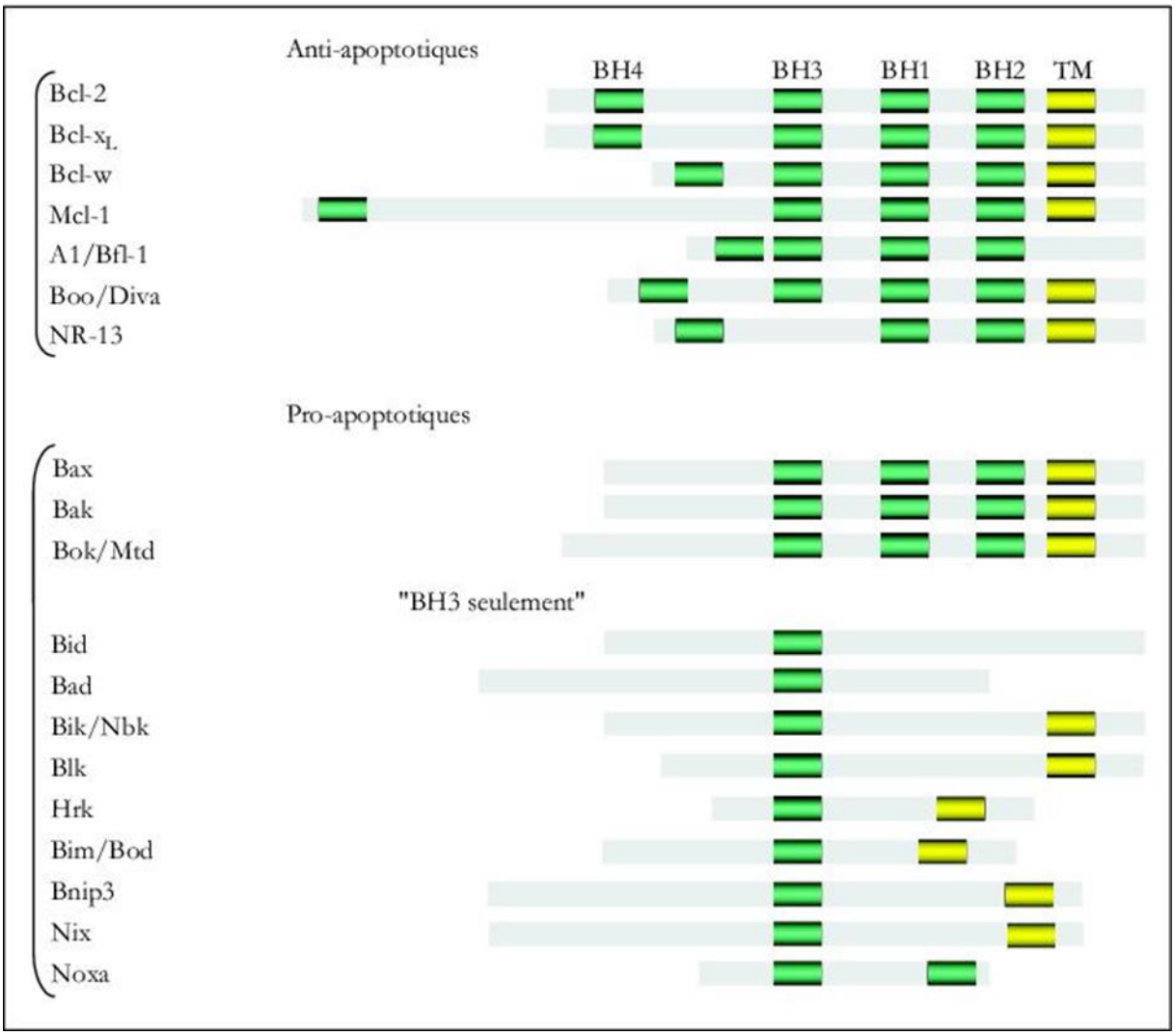


Figure 25: structure of proteins of the Bcl-2 family

4.4 Caspases

Caspases, or cysteinyl-aspartate-cleaving proteases, are key cysteine proteases in the processes of apoptosis, necrosis, and inflammation. Their role in cell death has been highlighted by identifying the pro-apoptotic ced-3 gene in the nematode *C. elegans*, whose first mammalian counterpart, the ICE (interleukin-1 beta converting enzyme) gene, has also been identified. These enzymes cleave proteins at specific sites through a conserved catalytic site containing a cysteine residue and a QACXG-like peptide sequence. To prevent unregulated apoptosis, caspases exist in an inactivated form, called procaspases, in the cytoplasm. When activated, they can cleave other procaspases, resulting in cascading activation.

The structure of caspases includes a variable N-terminal prodomain and two subunits (large: 17-21 kDa; small: 10-14 kDa) formed after cleavage. Initiating caspases, having a longer

prodomain, have domains such as the CARD domain (for caspases 2 and 9) or the DED domain (for caspases 8 and 10), necessary for their activation and that of effector caspases.

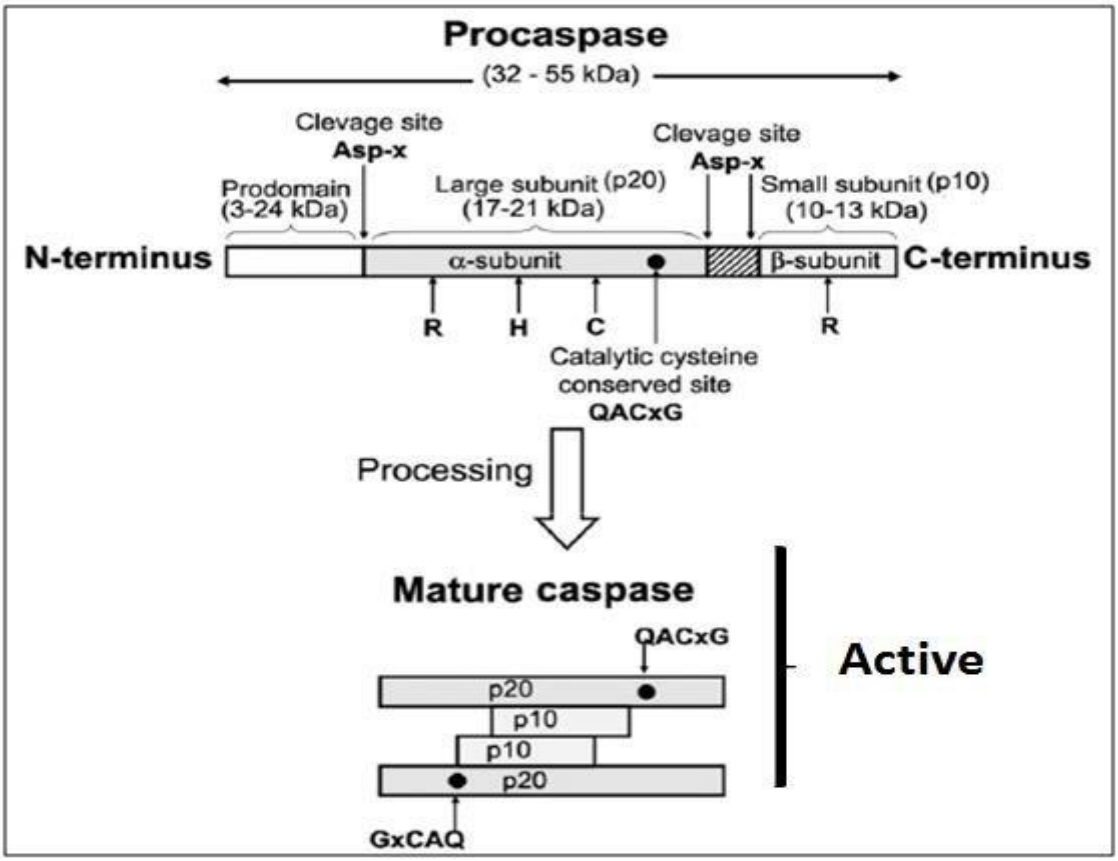


Figure 26: structure and activation of caspases

Caspases primarily target various proteins within the cell to orchestrate the process of apoptosis and other cellular pathways, such as endonucleases, cell integrity proteins (cytoskeleton proteins), regulatory proteins, or even anti-apoptotic proteins.

To date, 15 caspases have been described, including caspases 4 and 5, pro-inflammatory, and initiating caspases (1, 2, 8, 9, 10, 12) which are active in monomeric form, while effector caspases (3, 6, 7) assemble into active dimers.

Schematically, this cell death takes place according to a series of ordered steps:

- an initiation phase
- a decision stage, where the death process begins and cannot be stopped
- an execution phase
- a phase of phagocytosis.

In this case, the cell is "ordered" to die, i.e., to set its program of self-destruction in motion. The apoptotic signal can come from the outside, i.e., from the cellular environment (extrinsic pathway) or from within the cell itself (intrinsic pathway).

4.5 The intrinsic (mitochondrial) pathway

Members of the Bcl-2 family regulate the intrinsic pathway of apoptosis, and its effect is mediated by the release of proteins such as cytochrome c, which occurs due to the permeabilization of the outer mitochondrial membrane. Death signals can act directly or indirectly on the mitochondria, forming the apoptosome complex () (Figure). Pro-apoptotic proteins of the Bcl-2 family induce the release of mitochondrial apoptogenic factors by regulation of mitochondrial permeabilization such as cytochrome c, as well as caspases, IAPs (caspase inhibitors), and the mitochondrial caspase activator (Smac) and Omi, which negatively regulate IAPs. There are also caspase-independent mechanisms within the intrinsic pathway involving the release of two proteins: apoptosis-inducing factor (AIF) and endonuclease G (EndoG).

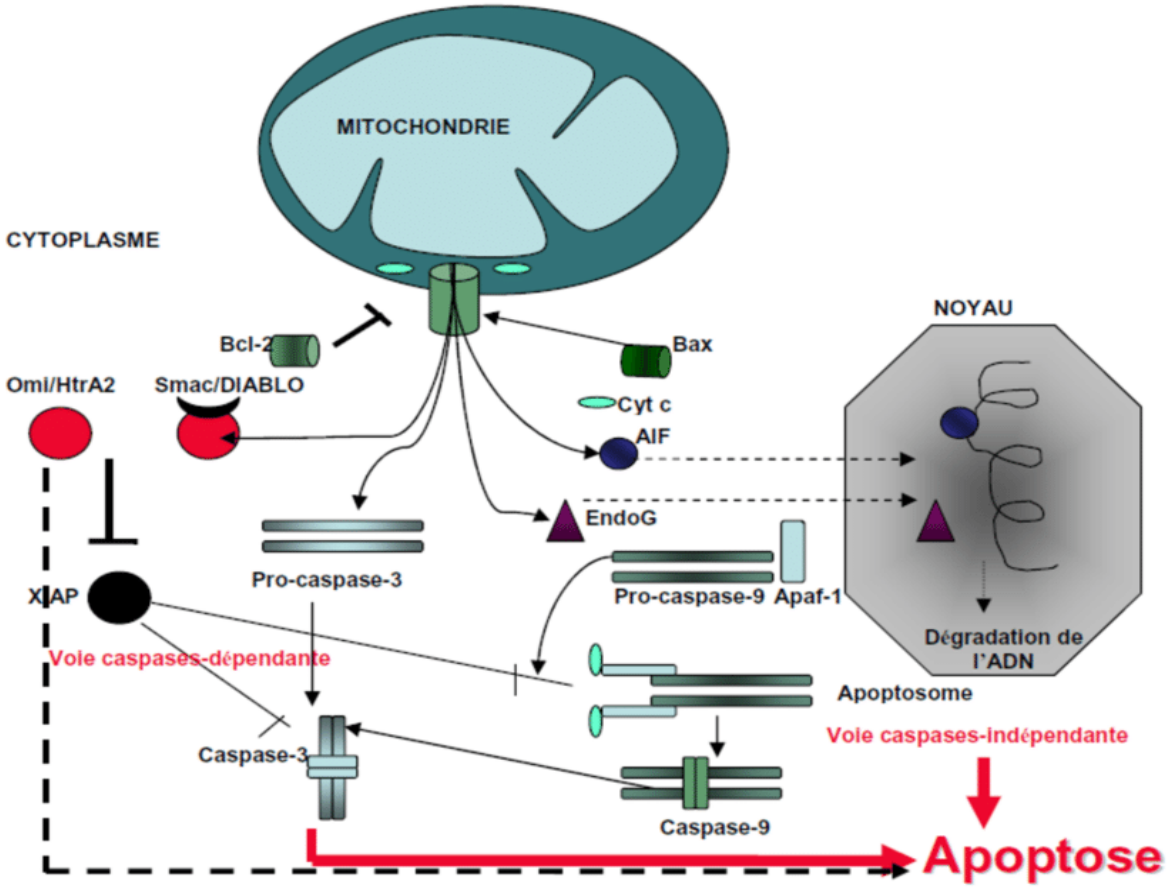


Figure 27: shematic representation of intrinsic pathway of apoptosis

During the initiation phase of apoptosis, second messengers accumulate in the cell, increasing the permeability of mitochondrial membranes. These second messengers are associated with the response to disruptions in cellular homeostasis. Membrane permeabilization is mediated by a heterogeneous set of factors, including non-protein and protein agents. However, two groups of proteins play a key role: on the one hand, the anti-apoptotic proteins of the Bcl-2 family, such as Bcl-2 and Bcl-XL, which are located in the outer mitochondrial membrane and inhibit the permeabilization of this membrane; on the other hand, pro-apoptotic proteins, such as Bax, Bad, Bid, Bim, and Bak, which, in response to apoptotic signals, integrate into the outer mitochondrial membrane to form oligomers and neutralize anti-apoptotic proteins, thus contributing to the formation of pores. The ratio between pro-apoptotic and anti-apoptotic molecules regulates membrane permeability and determines the cell's engagement with the apoptotic program (Figure). It should also be noted that some viral proteins, such as viral hepatitis protein X and bacterial proteins, can directly influence the permeability of mitochondrial membranes.

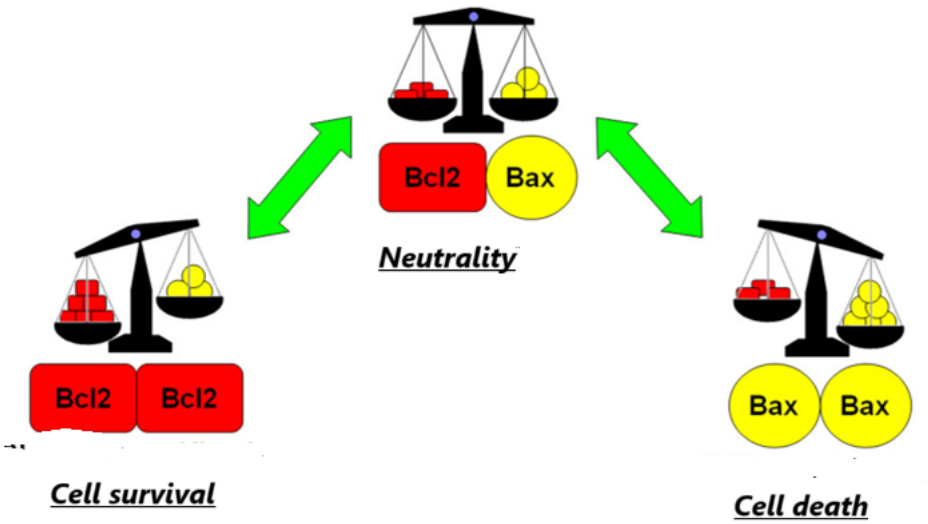


Figure 28 : apoptotic balance

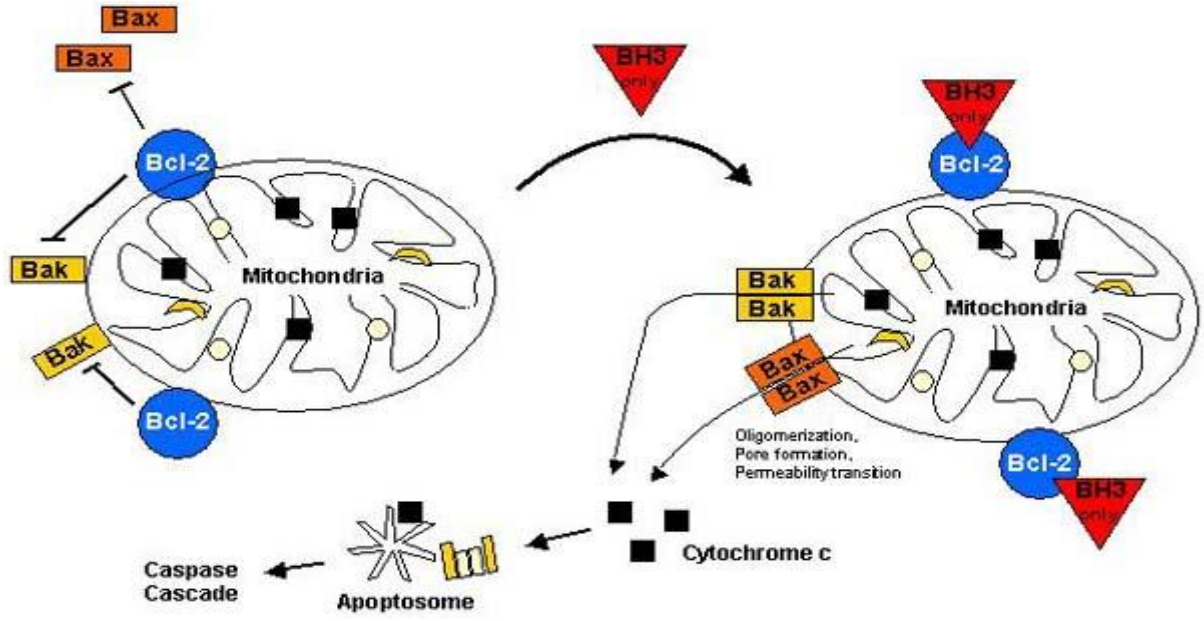


Figure 29: Mechanism of Apoptosis Regulation via Bcl-2 Family Proteins in Mitochondria

At the same time, the activation of catabolic hydrolases, mainly caspases, is also involved in the apoptotic process. Caspases are apoptosis-specific cysteine proteins that cleave their substrates at the level of aspartate residues. Their activation results from the passage of cytochrome c from the intermembrane space to the cytosol, facilitated by the permeabilization of the outer membrane. Upon leaving this space, once cytochrome c is released, it binds to apaf-1 (apoptosis protease activating factor-1), and this interaction promotes the self-assembly apaf-1 into a wheel-shaped complex. This complex then recruits pro-caspase-9, thus forming the apoptosome (figure) and allowing cleavage and activation in caspase-9. This triggers a cascade of caspase activation with proteolytic activity. The apoptosome, therefore plays a crucial role in initiating the caspase activation cascade, ultimately leading to the degradation of cellular components and the activation of the apoptotic program.

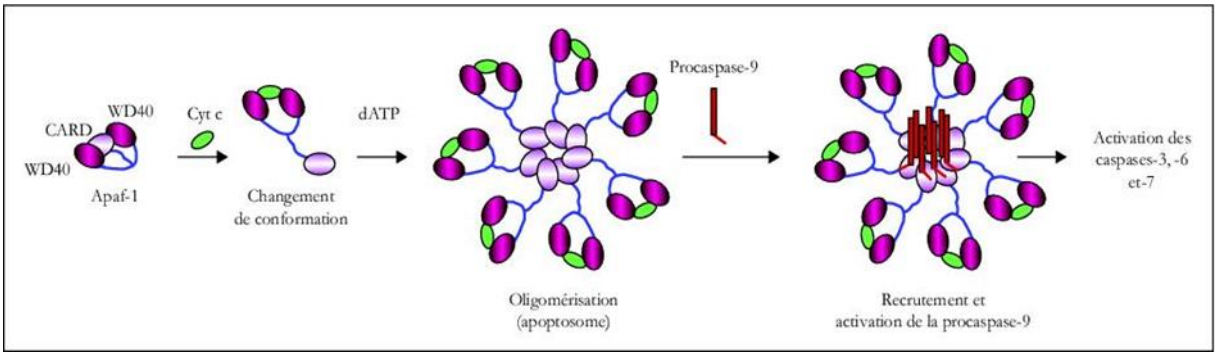


Figure 30: molecular structure of apoptosome

Different hypotheses make it possible to model this permeabilization of the outer membrane of the mitochondria (figure).

- **-Modulation of the opening of existing channels**: this first model postulates the opening by Bax of a mega-channel called the permeability transition pore (PTP) (Figure a). Alternatively, Bax would interact with VDAC (voltage-gated anion channel), resulting in a significant confomational change, causing the pore to open, and allowing the escape of pro-apoptotic effectors (Figure b). The electrophysiological characterization of the currents generated in the outer membrane of the mitochondria under the action of Bax also made it possible to discover the existence of an ion channel called MAC (Mitochondrial Apoptosis-induced Channel), different from the PTP, whose opening would also be modulated by Bax.
- **-Channel formation by pro-apoptotic proteins of the Bcl-2 family**: This model is based on Bax's ability to form channels, particularly in synthetic phospholipid membranes. Bax can associate oligomers of different sizes to form channels wide enough to release cytochrome c. The oligomerization of Bax seems to be an irreversible process; the formation of a heterodimer with tBid, a form activated by proteolysis of Bid, induces a conformational change of Bax, allowing it to associate with the mitochondrial membrane to induce its permeabilization and to lead to apoptosis. A similar process is envisaged for the mitochondrial protein Bak.
- **Fission mitochondriale** Fission events participate in the morphology and "natural" multiplication of mitochondria. Nevertheless, a division of the mitochondria into small punctiform organelles was observed during MCP. The Dynamin-Related protein 1 (Drp-1) is involved in splitting the outer membrane (Figure 14 e). The inhibition of fission does not allow the formation of punctiform organelles, the fall of the $\Delta\Psi m$, and the release of cytochrome c, thus blocking the PCM. This suggests that mitochondrial fission could be involved in releasing death effectors. Bax would partner with Drp-1 to fragment the mitochondria.

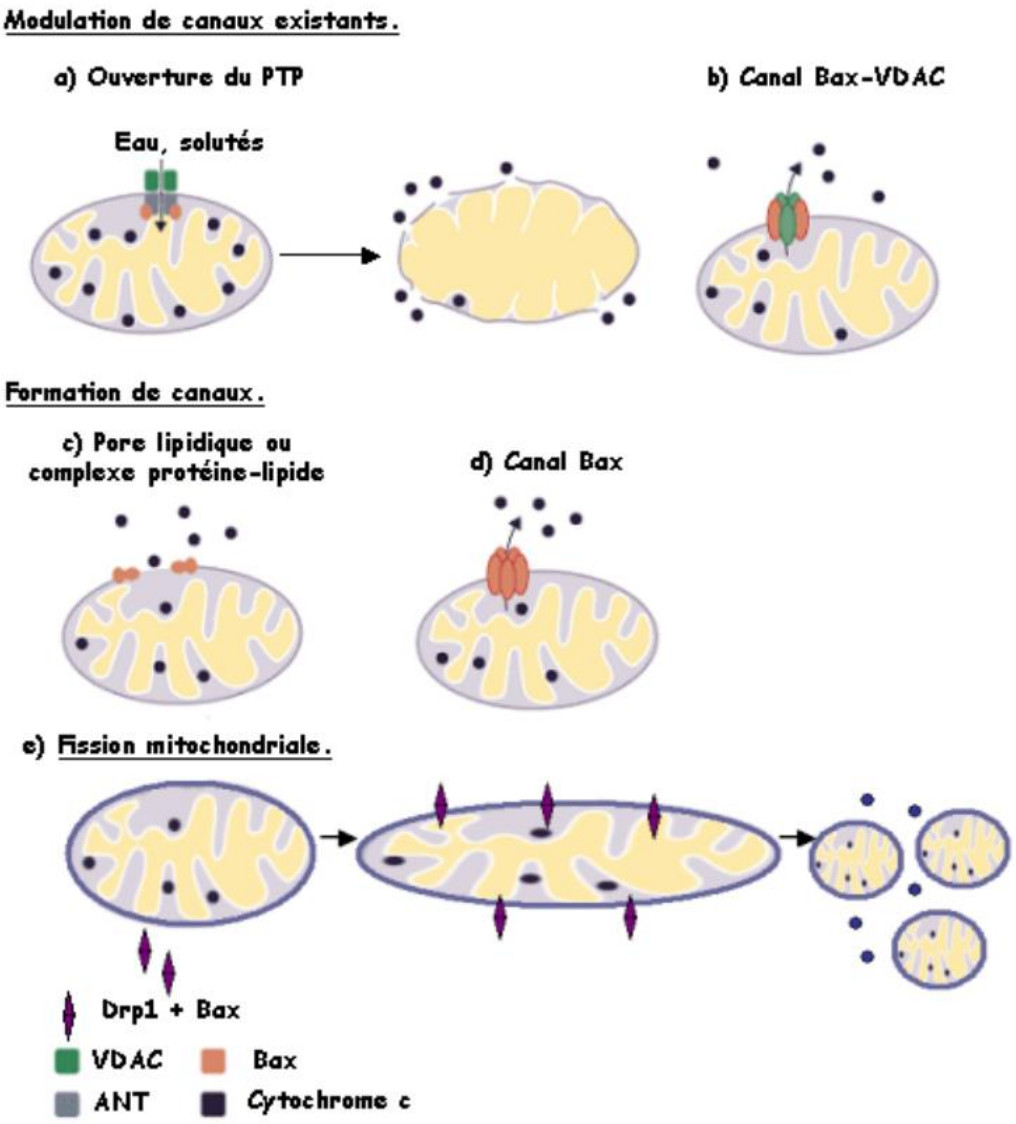


Figure 31: Different models proposed for mitochondrial release of cytochrome c. During cell death, mitochondrial factors are released into the cytosol. In models a) and b), the opening of existing channels is modulated. Model a) engages the opening of the permeability transition pore (PTP), the swelling of the mitochondrial matrix induces the rupture of the outer membrane and allows the extramitochondrial release of pro-apoptotic factors, while model b) engages the cooperation of Bax and VDAC (voltage-gated anion channel) to release apoptotic effectors. In models c), d), a large channel is formed in the outer membrane of the mitochondria and allows the release of mitochondrial factors, and finally, model e) is based on mitochondrial fission induced by the proteins Drp-1 (Dynamin-related protein 1) and Bax.

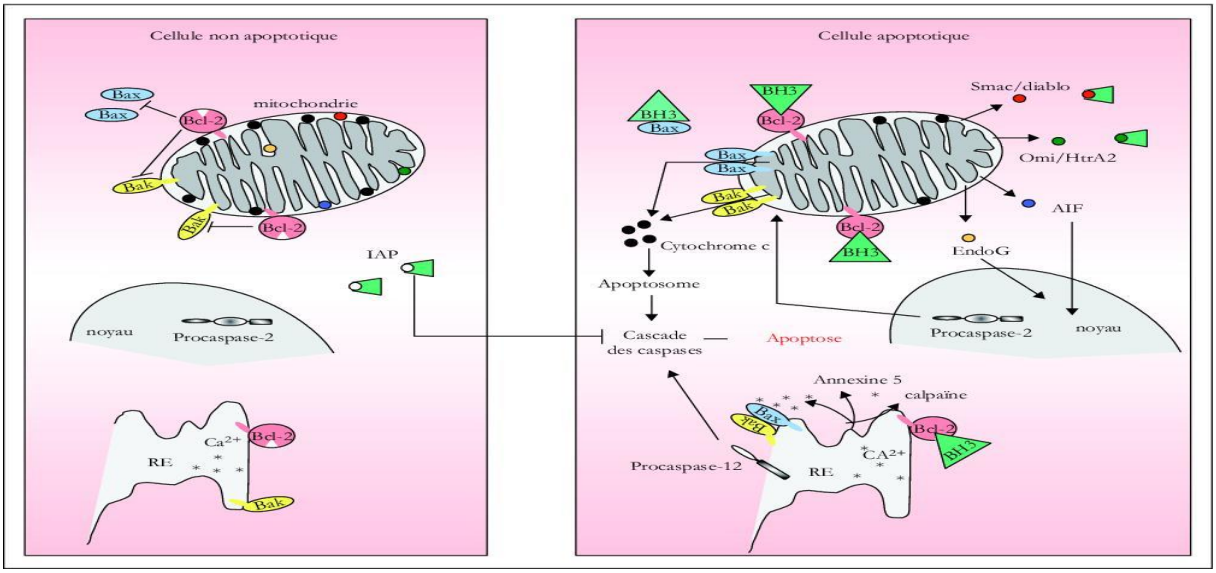


Figure 32 : Comparison of Non-Apoptotic and Apoptotic Cell Mechanisms Involving Mitochondrial Pathways

Another apoptosis pathway independent of caspase activation involves the release of an intermembrane protein, AIF (apoptosis-inducing factor), which binds to DNA after translocation to the nucleus to induce chromatin condensation.

4.6 The extrinsic pathway

The extrinsic apoptosis pathway is an essential mechanism that initiates cell death in response to signals from outside the cell. This process begins with the activation of death receptors on the surface of cells, such as the Fas receptor (CD95), which binds to its FasL ligand, expressed by immune cells. Other receptors, such as the tumor necrosis factor receptor (TNF-R), bind to TNF-α, a pro-inflammatory cytokine, and the TRAIL receptors, which interact with the TNF-related apoptosis-inducing ligand (TRAIL). When the ligand binds to the receptor, it leads to oligomerization of the receptor, a crucial event for activating signaling pathways.

An initiation complex called Complex I or DISC is formed following the receptors' activation. The DISC consists of the activated receptor, adaptor proteins (such as FADD), and the information needed to recruit and activate caspases, including caspase-8.

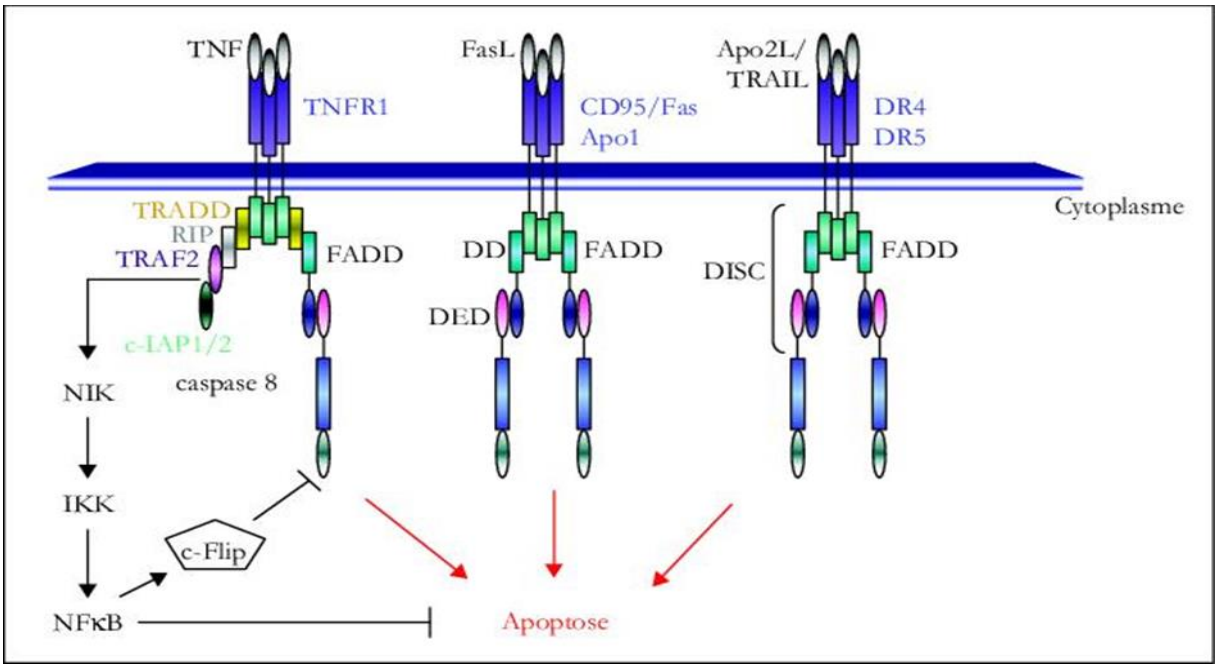


Figure 33: signaling pathways through "domain of death" receptors. The trimerization of the receptors of Fas (also called Apo1 or CD95), TNF (TNFR1) or TNF-related apoptosis-inducing ligand (TRAIL) (DR4 and DR5) allows the recruitment of different adaptor proteins that will activate distinct signaling pathways (c-Flip (cellular FAD-like ICE inhibitory protein) inhibits the formation of DISC (Death-Inducing Signaling Complex) by preventing the recruitment of procaspase 8. (TNFR-associated factor (TRAF), TNFR-associated death domain (TRADD), and Fas-associated death domain (FADD)).

The latter is recruited to the complex by its specific domains, and once it arrives, it is activated by self-cleavage. The activation of caspase-8 then triggers a signaling cascade that activates other caspases, especially effector caspases such as caspase-3, caspase-6, and caspase-7. These effector caspases play a key role in executing apoptotic events by cleaving various proteins, thereby causing the degradation of cellular structures, inhibiting anti-apoptotic proteins, and the activation of endonucleases that contribute to cell death.

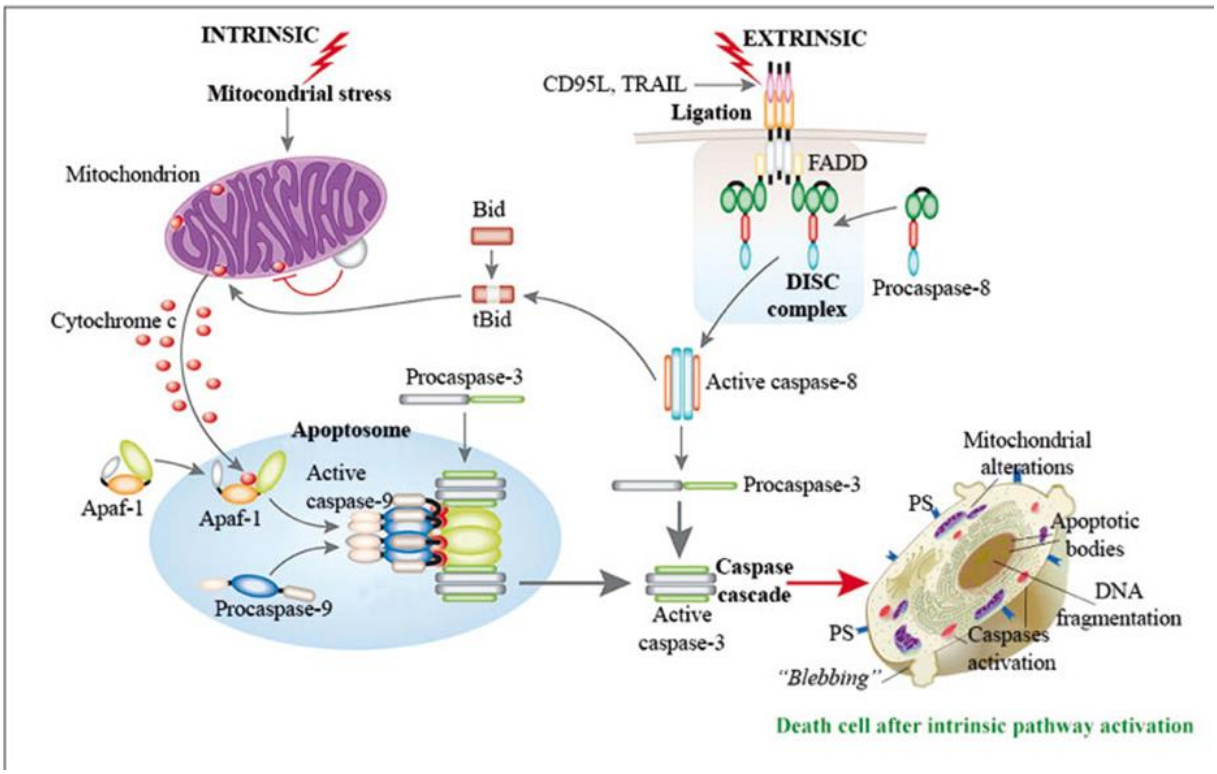


Figure 34 : apoptotic pathway, the extrinsic pathway involves so-called death receptor (CD95, TRAIL), the intrinsic one involves mitochondrial granules.

The effects of activating both pathways manifest themselves in morphological changes characteristic of apoptosis, including chromatin condensation, DNA fragmentation, and the formation of apoptotic bodies (figure), which can be eliminated by immune cells. This mechanism is crucial in various contexts, such as the immune response, removing infected or damaged cells, and embryonic development, where certain cells are eliminated for proper growth. However, some cancer cells and viruses can counteract this process by expressing inhibitory molecules, such as apoptosis inhibitor proteins (APIs), or reducing the expression of death receptors.

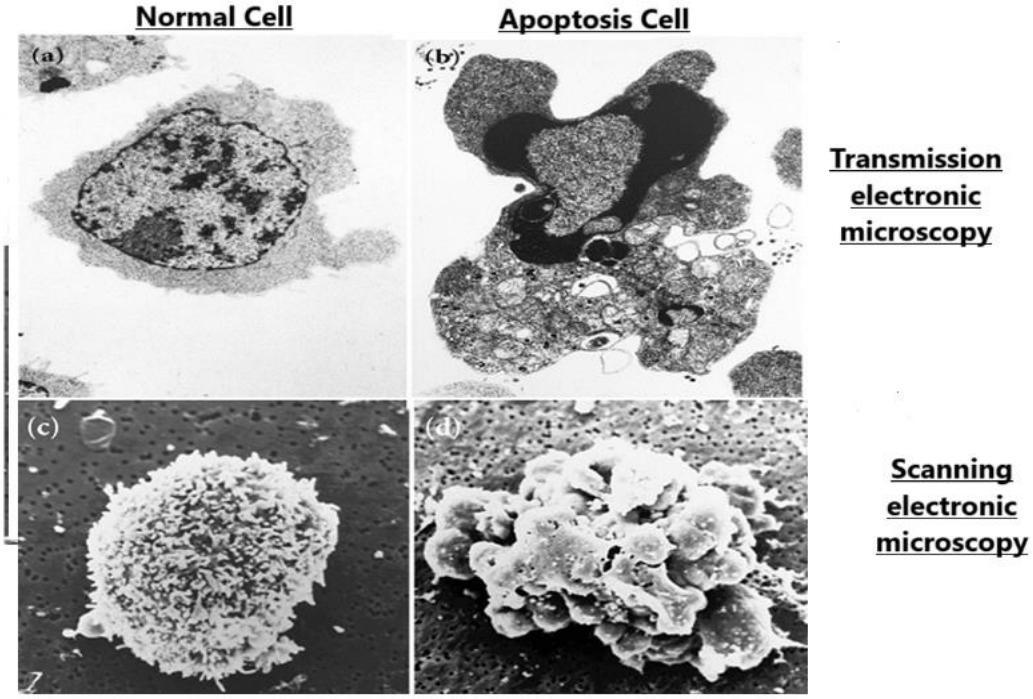


Figure 35 : Microscopic observation of normal and apoptotic cell

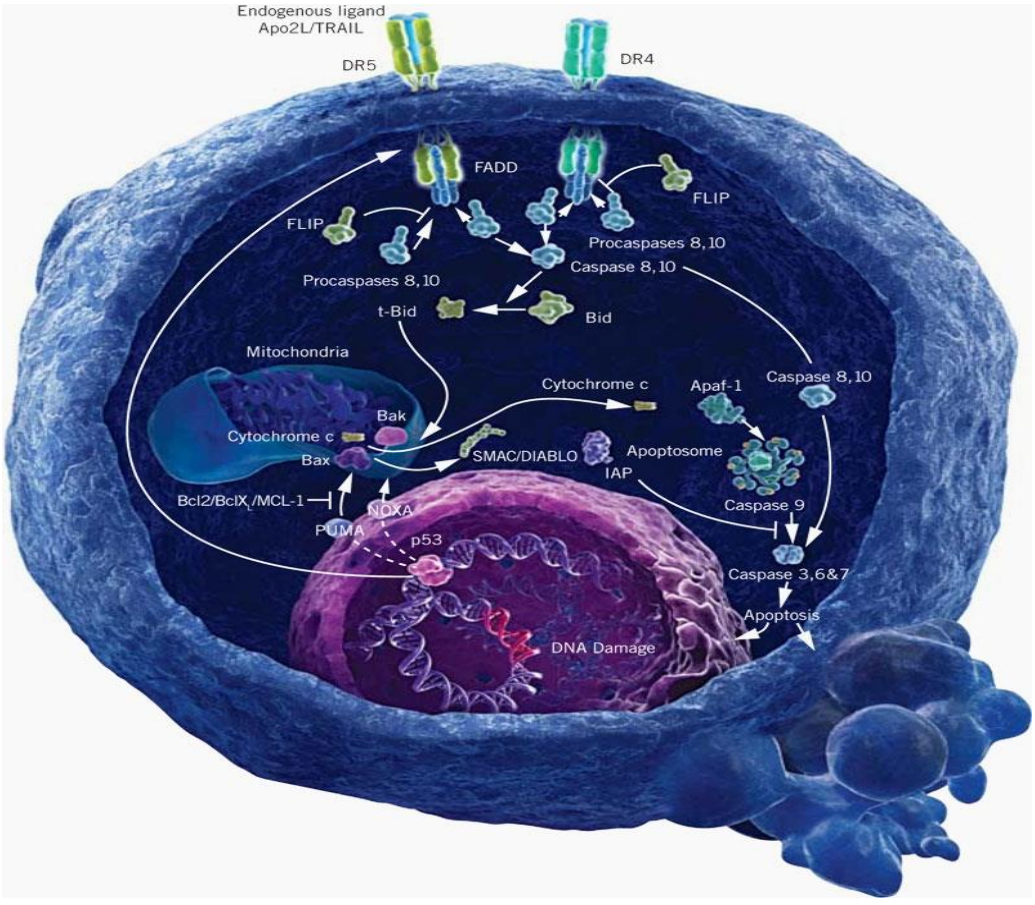


Figure 36: representation of apoptosis at the cellular level

4.7 The caspase-2-dependent pathway

Caspase-2 is an initiating caspase essential for the apoptosis process, although its mechanism of action is less well-known than other caspases (8 and 9) (figure). Its activation can occur due to various cellular stresses, such as DNA damage or oxidative stress, and it can be involved in extrinsic and intrinsic signaling pathways. One of the key complexes for its activation is the PIDDosome, which forms in response to DNA damage and includes the PIDD (p53-induced protein with a death domain) protein associated with RAIDD and caspase-2, thus promoting its cleavage into an active form (figure). Once activated, caspase-2 plays a central role in apoptosis by cleaving specific substrates and activating other caspases, such as caspase-3. In addition to its apoptotic function, caspase-2 is involved in cell cycle control and stress response. Its regulation is complex and depends on various factors, including anti-apoptotic proteins that can inhibit its activation. Overall, caspase-2 represents a key component of apoptotic signaling, and understanding it may have important implications in developing therapies targeting apoptosis in diseases such as cancer.

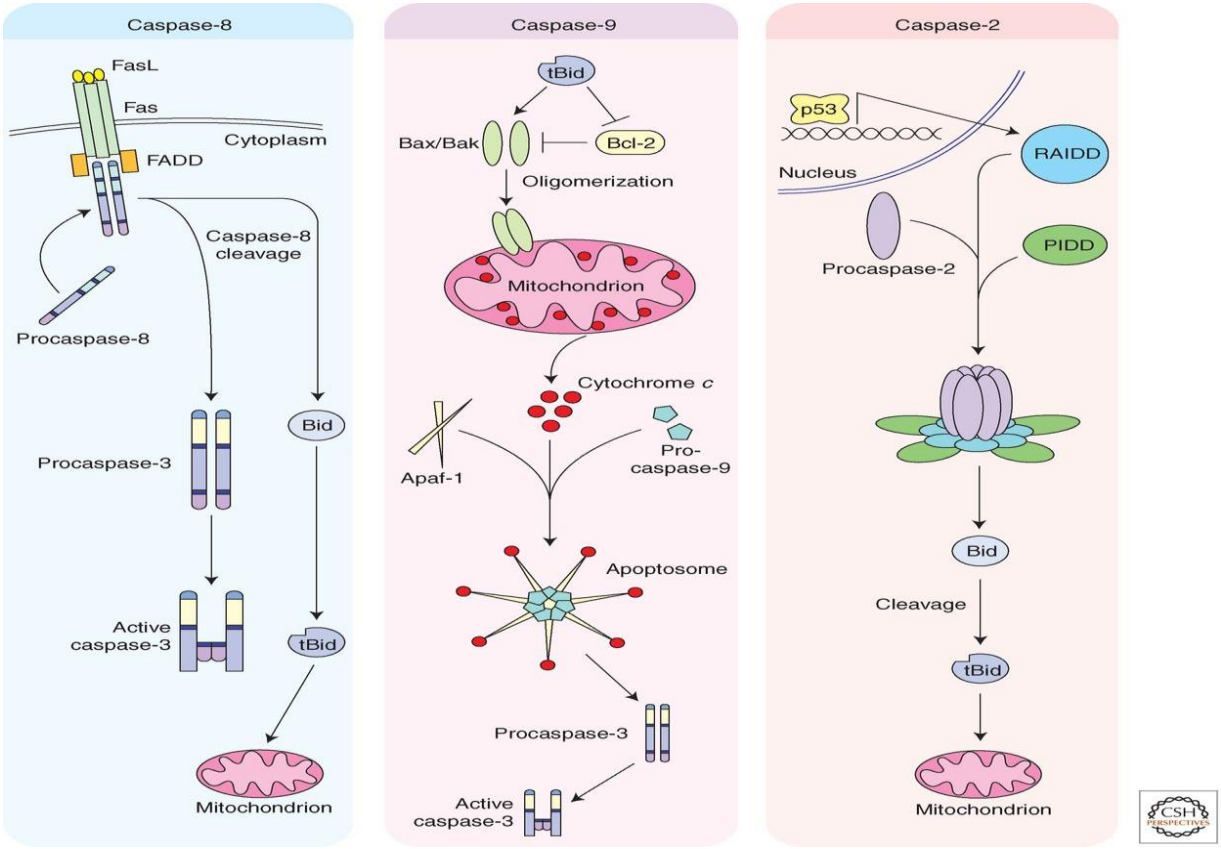


Figure 37 : Caspase Activation Pathways in Apoptosis: Caspase-8, Caspase-9, and Caspase-2 Mechanisms

4.8 Caspase-independent pathway (Granzyme pathway)

Granzymes A (GrA) and B (GrB) play crucial roles in inducing cell death, especially during the immune response against infected or tumor cells. Research has shed light on the mechanisms of action of granzyme A (GrA) and granzyme B (GrB), two key proteins secreted by cytotoxic T cells and natural killer (NK) cells. Although these two enzymes are involved in cell death, they act via distinct mechanisms.

GrA, a caspase-independent cellular degradation pathway, enters the cytosol of the target cell through pores formed by perforin in the presence of calcium. Once released, GrA generates pseudo strands of single-stranded DNA and induces apoptosis by activating the endonuclease GAAD (GrA-activated DNase). GAAD activity is regulated by an inhibitory complex in the endoplasmic reticulum, released when GrA cleaves specific proteins.

On the other hand, GrB also penetrates target cells via perforated pores and induces apoptosis mainly through the direct activation of caspases. It cleaves various cytosolic proteins, thus causing DNA fragmentation and morphological changes characteristic of apoptosis. GrB can also activate signaling pathways that enhance immune responses. Understanding the complementary roles of GrA and GrB in eliminating infected or tumor cells highlights their importance in the immune response and opens up prospects for new immunological therapies.

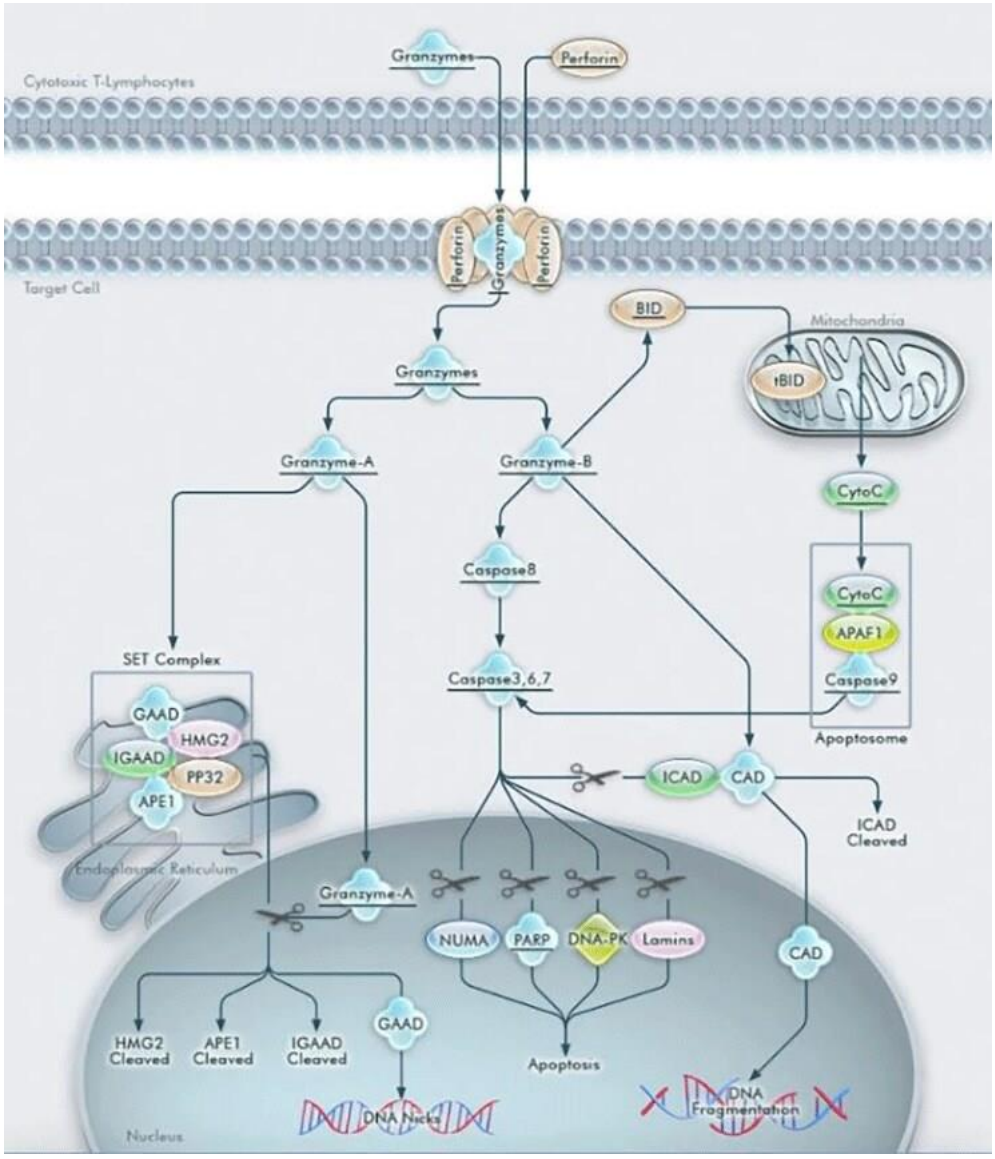


Figure 38 : Granzyme pathway

4.9 Apoptosis dysfunction

As tumor cells can alter signaling pathways that regulate apoptosis, including by overexpressing anti-apoptotic proteins like Bcl-2 and inhibiting pro-apoptotic proteins like Bax. This results in resistance to apoptosis by preventing the activation of apoptotic cascades.

Tumor cells can also decrease the expression of death receptors, such as Fas (CD95), or ligands capable of inducing apoptosis, thereby reducing their sensitivity to apoptotic signals.

5 THE ACQUISITION OF IMMORTALIZATION.

5.1 Telomeres, structure and roles

Telomere comes from the Greek words meaning "the end" (telos) and "the part" (meros). Telomeres are highly repetitive regions of DNA located at the end of each linear chromosome.

Telomeres, complex and dynamic constituents of DNA, are positioned as guardians of genomic integrity by playing a critical role in protecting advanced chromosomes from enzymatic end-breaking and chromosome fusion. They are made up of G-rich tandem repeats (5'-TTAGGG-3') in mammals over a length ranging from 3 to 20kb and a protein set protecting this DNA, forming the complex called shelterin (shelter) or telosome (figure).

Among telomeric proteins, a set of 6 proteins called telosome or shelterin complex plays a major role in telomeric structure and function. This telosome includes the proteins POT1 (Protection of Telomeres 1), TRF1, TRF2 (Telomeric Repeat binding Factor 1 and 2), TIN2 (TRF1-Interacting Nuclear protein 2), RAP1 (Repressor/Activator Protein 1, also known as TERF2IP for telomeric repeat binding factor 2, interacting protein) and TPP1 (TIN2 and POT1 interacting Protein). This structure is crucial for genomic stability, coding DNA protection, and cellular function maintenance.

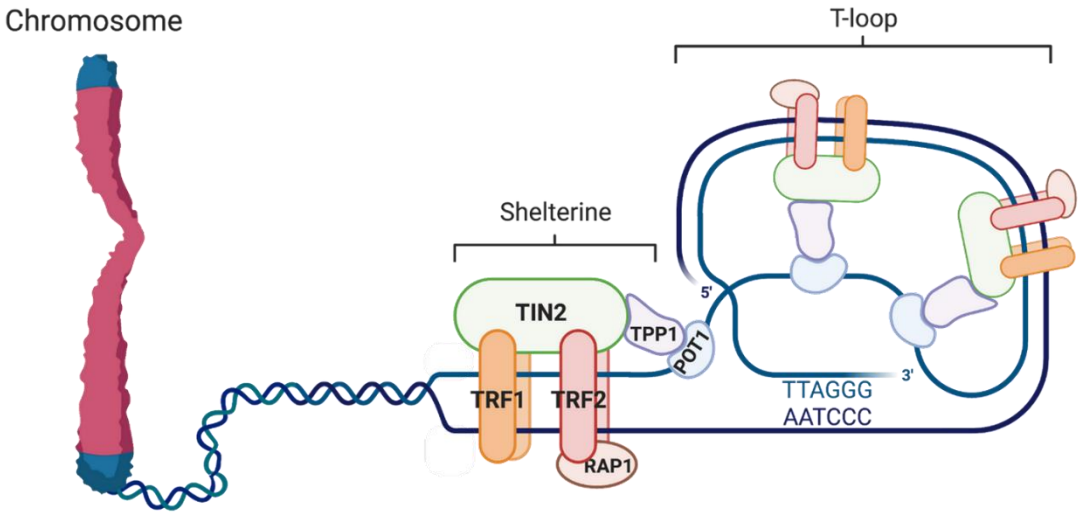


Figure 39: structure of telomeres

These proteins, specifically located at the telomere level, are present during all cell cycle phases and have no other function in the nucleus. The recognition of TTAGGG explains the specificity of the shelterin complex for telomeric DNA repeats by 3 components: TRF1, TRF2, and POT1. This is because TRF1 and TRF2 bind directly to double-stranded DNA; POT1 binds to the single-strand sequences of the free 3' end and loop D. TIN2 binds TRF1, TRF2 and TPP1 while TPP1 binds TIN2 and POT1. RAP1 is only linked to TRF2. These proteins form a stable complex, even in the absence of telomeric DNA; nevertheless, the components of the telosome are quantitatively associated with telomeres, their assembly being a function of the number of TTAGGG repeats. In mammals, the single-stranded telomere region can invade the double-stranded regions of the same telomere with a D-loop favored by shelterin proteins (Figure). This structure, observed by electron microscopy, is called a “t-loop" and is proposed as an additional mode of telomere protection.

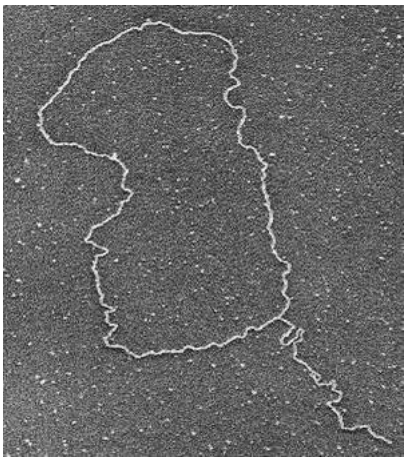


Figure 40: image of a T-loop telomeric loop visualization by electron microscopy

Shelterin proteins play a crucial role in the protection and regulation of telomeres. They prevent telomeres from being recognized as DNA breaks, thus preventing the activation of cellular repair pathways. Shelterin also maintains telomere structure by forming protective loops (T-loops) and downregulating telomerase activity to maintain adequate telomere length. In addition, these proteins are involved in signaling telomere states, contributing to the cellular response to short telomeres, which can induce senescence.

Indeed, at each cell division, during DNA replication, the DNA polymerase enzyme complex cannot copy the last nucleotides of the chromosomal ends, making a shortening of this structure inevitable (the end replication problem) (figure). Indeed, when replication reaches the end of a chromosome, no additional strand is left to serve as a template after synthesizing the last

Okazaki fragment. Therefore, DNA polymerase cannot complete the synthesis of this segment at the 3' end. As cells divide, this forgetting of the end means that the telomeres' length decreases with each replication. The shortening of human telomeres is about 100 to 150bp per cell division and can be accelerated by oxidative stress and exonucleases.

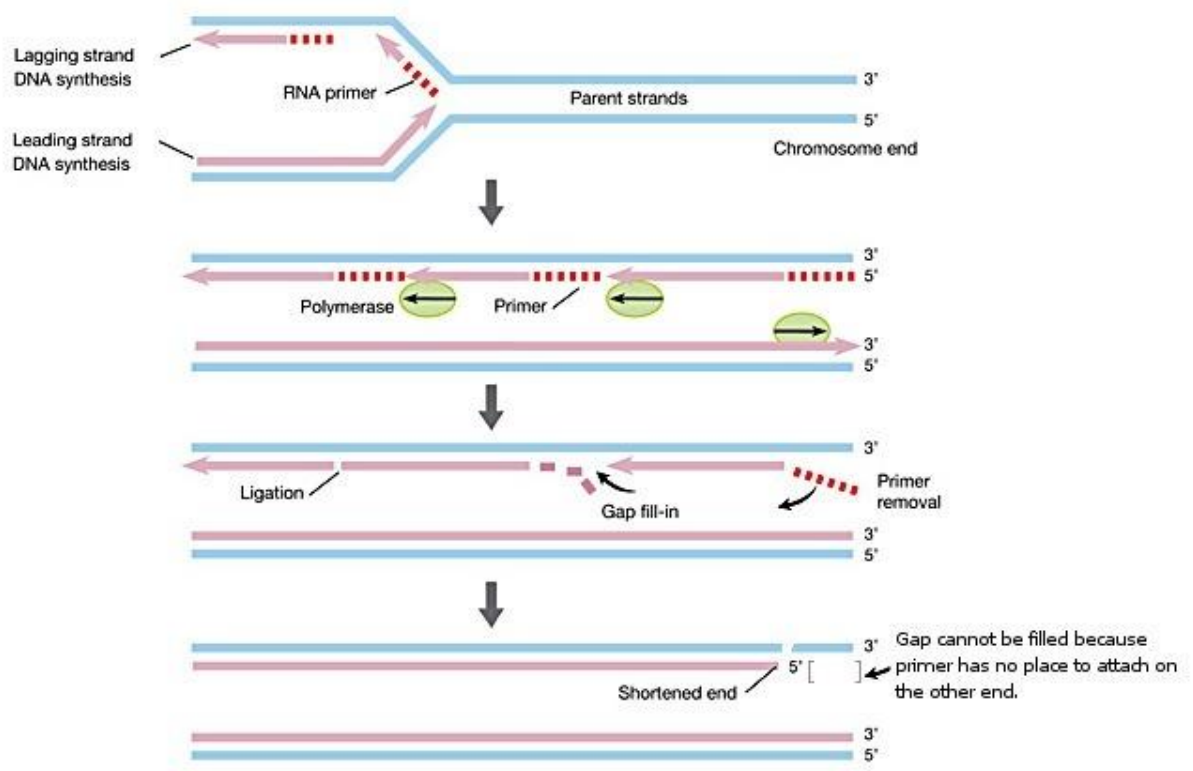


Figure 41: the end replication problem

Beyond a certain limit, this shortening leads to the cellular phenomena of replicative senescence or apoptosis, which involves the anti-oncogenes p53, p16INK4a, and Rb and trigger cell death. Indeed, the shortening of telomeres triggers cellular senescence. When telomeres become short, they lose their ability to form the protective T-loop structure. This then exposes the ends of the chromosomes, which are detected by the cell as DNA damage and sends signals that activate the ATM protein, leading to Chk1/2 and eventually p53. This activation of p53 initiates cellular responses that inhibit the cell cycle (transcription of P21 and inhibition of pRb), preventing cells from dividing further and thus resulting in senescence (Figure) as it induces the triggering of apoptosis (transcription of BAX, PUMA, etc.). This mechanism serves as a protection against uncontrolled cell proliferation and genetic damage.

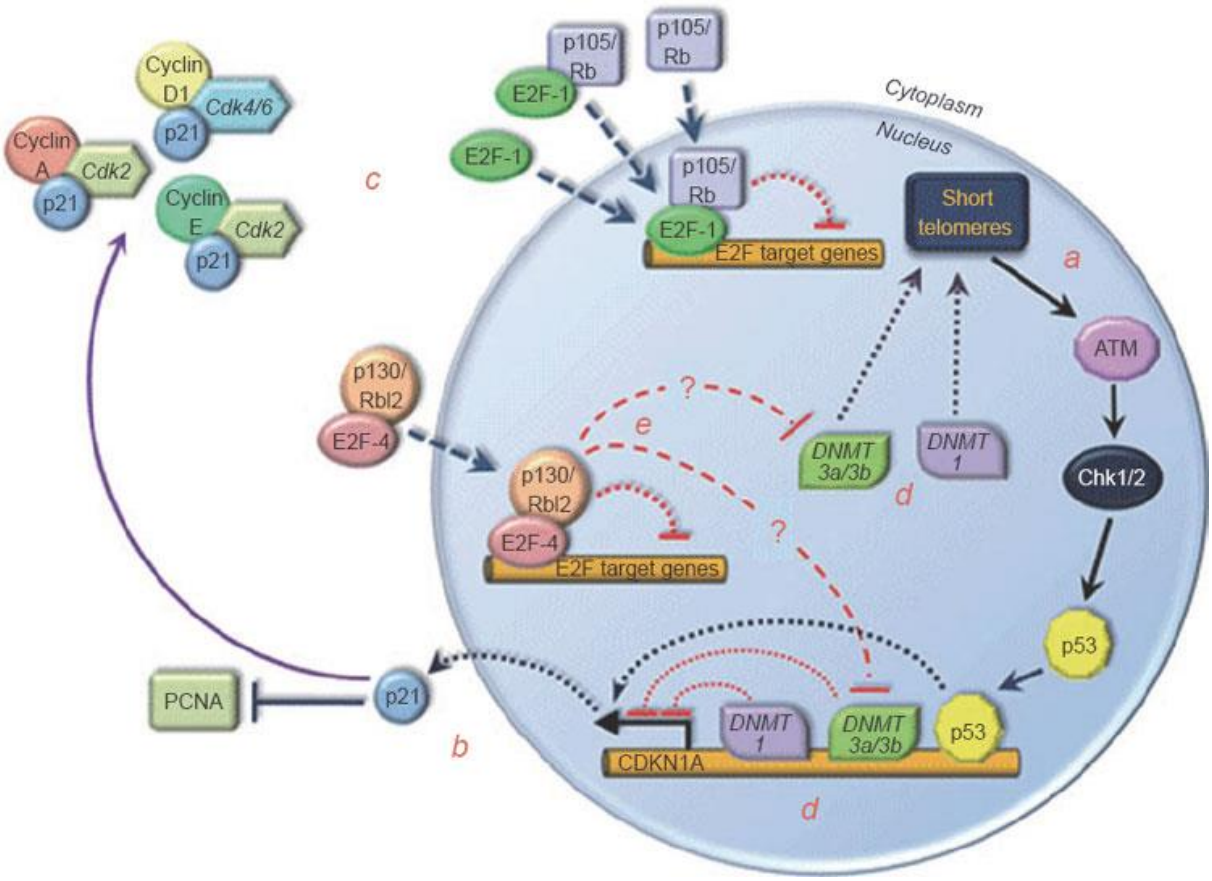


Figure 42: triggering of cellular senescence by telomere shortening

In these cells, telomeres can, therefore, become very short and send a signal to the cell to stop cell proliferation. Telomeres, hence, also serve as a molecular clock to count the number of cell divisions since the loss of telomerase and as an alarm so that the cell stops proliferating, thus increasing the number of cell divisions (Hayflick limit). Thus, the homeostasis of many human organs depends on the proper functioning of telomeres and their shortening properties. This balance is broken in all cancer cells without exception. These bypass apoptosis by lengthening telomeres through telomerase activation (Figure).

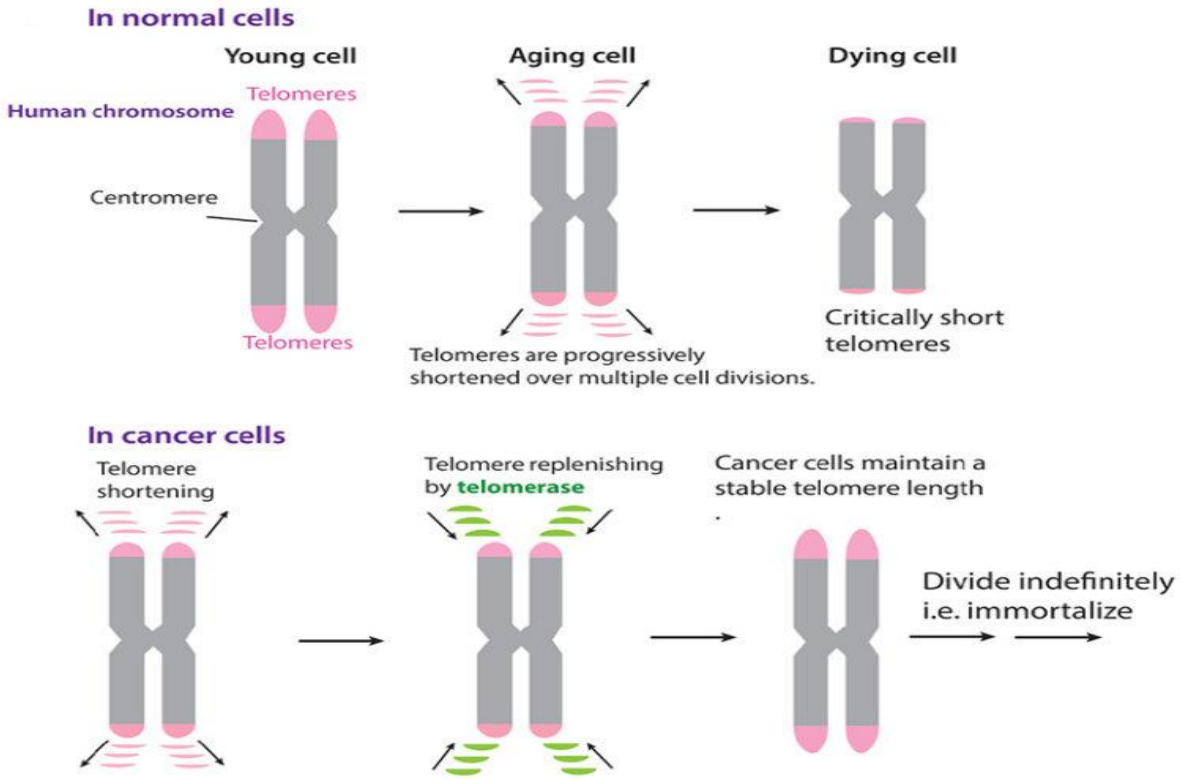


Figure 43: effect of telomerase activation on embryonic and cancer cells

5.2 Telomerase

It is a 1000 kDa protein in humans. It comprises two main sub-units. A catalytic subunit which is a reverse transcriptase (TERT) highly conserved in unicellular and multicellular organisms and a subunit that corresponds to an RNA sequence, the TERC (Telomerase RNA Component), also referred to as TR or TER) which contains the AAUCCC sequence (in humans) (Figure). These two subunits are the minimum essential elements for telomerase activity.

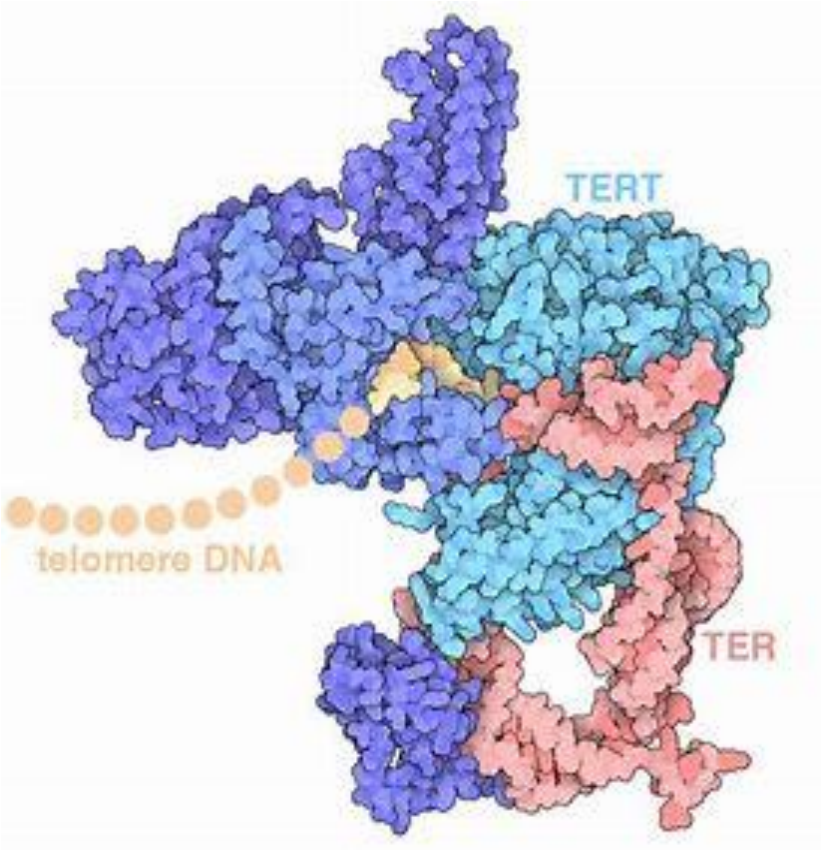


Figure 44 : structure of telomerase.

5.3 How telomerase works

Telomerase elongates telomeres' 3' terminal end by positioning itself at a recognition site at the telomere end via a TERC matrix site (matrix). The telomere binding site is a short RNA matrix sequence characteristic of each cell type (CAAUCCCAAUC in humans) at the level of the CR1 domain. The resulting extension of the telomere corresponds to the addition of a telomere repeat at its 3' end. After elongation, the neoformed C-strand is normally used to synthesize double-stranded telomeric DNA.

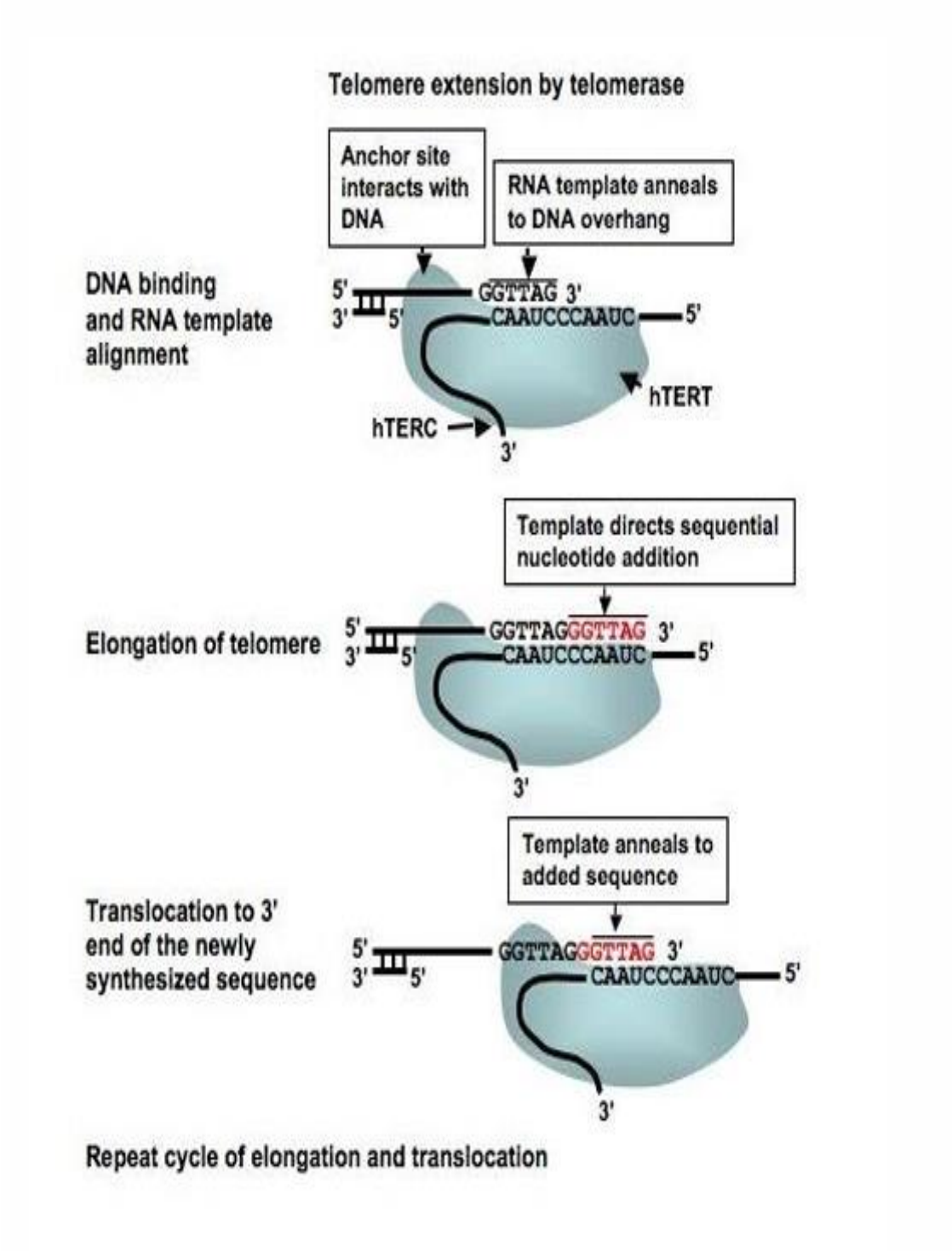


Figure 45: Shows the steps of telomere elongation by telomerase

5.4 Regulation of telomerase by shelterin proteins

Tankyrase 1 (TANK1) is a protein of the poly(ADP-ribose) polymerase (PARP) family that modulates the activity of TRF1. The poly(ADP-ribosylation of TRF1 inhibits the binding of this factor to telomeres. In addition, an overexpression of TANK1 in the nucleus of positive telomerase cells induces an increase in telomere size.

The enzymatic activity of TANK1 on TRF1 is regulated by the factor TIN2, which also contributes to the accumulation of TRF1 in telomeres. TRF1 negatively regulates telomerase by allowing the binding of POT1 to the single telomeric strand (G strand). Inactivation of TIN2 allows the poly(ADP) ribosyltransferase activity of TANK1 on TRF1, which decreases the affinity of the latter for double-stranded telomeric DNA and indirectly prevents the binding of POT1 to the telomeric single strand. The single strand then becomes accessible to telomerase (figure).

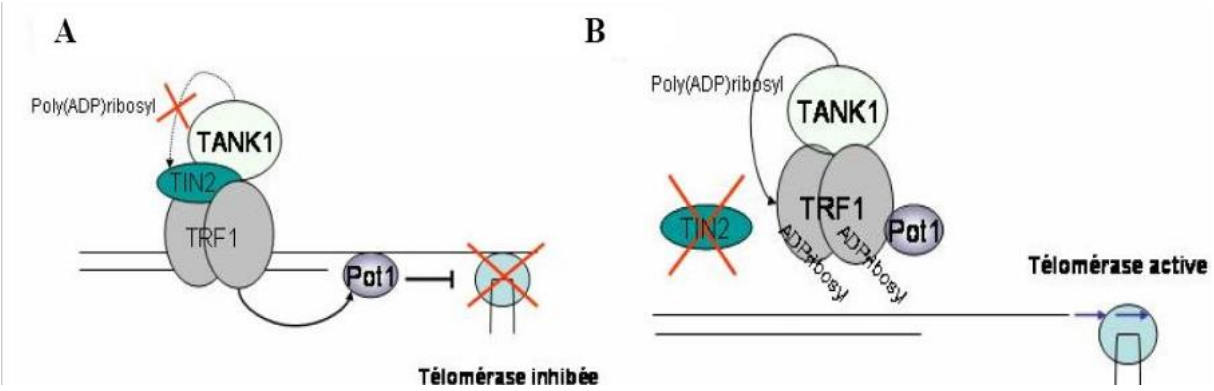


Figure 46: TIN2 regulator of tankyrase activity**.** A) TIN2 fixes and protects TRF1 from inactivation by tankyrase (TANK1). B) When TIN2 is inactivated, the poly(ADP) ribosyltransferase activity of TANK1 decreases the affinity of TRF1 for double-stranded telomeric DNA, which prevents the binding of POT1 to the telomeric single-strand. The single strand then becomes accessible to telomerase

Several mechanisms do the reactivation of telomerase by overexpression of the TERT gene by mutations introduced by new binding sites for transcription factors causing the increase of TERT expression, by amplification of the TERT gene within the cancer cell; by epigenetics with hypomethylation of the TERT promoter or by histone modification, by activation of oncogenes such as MYC which can activate the TERT gene, by alteration of tumor suppressors such as p53 and pRb or by amplification of certain signals such as cytokines or growth factors that can activate the pathways of TERT expression.

6 DEFINITION OF CANCER

6.1 Introduction

What is cancer? What are the causes of cancer? How does cancer grow and spread? Is cancer always hereditary? How is cancer diagnosed? Why can't our body neutralize it? How to treat cancer? How can cancer be prevented? These are all questions that can be asked and that we will try to answer in this chapter.

Cancer usually develops in several stages, ranging from an early stage (benign tumor) to an advanced stage (invasive tumor). These phases are often determined by the size of the cancer and the extent of the disease in the body.

6.2 Initiation Phase (Genotoxic)

This phase is characterized by rapid and irreversible DNA alteration following exposure to carcinogenic factors (which can be physical, chemical, or infectious). This alteration can manifest in mutations in one or more base pairs of DNA, insertions, deletions, chromosomal rearrangements, gene amplifications, or the activation of oncogenes. The cell is not yet cancerous at this stage but could become cancerous because its DNA has undergone alterations.

Different categories of initiators can be distinguished: chemical carcinogens, such as benzene, asbestos, and certain chemical agents present in food (such as aflatoxins), polycyclic aromatic hydrocarbons (PAHs) present in tobacco smoke, can form adducts with DNA, leading to structural changes in bases, such as guanine. These changes can generate errors during DNA replication if they are not repaired. Similarly, ionizing radiation, such as that emitted by X-rays, causes breaks in DNA strands, which, if not properly repaired by DNA repair mechanisms such as base excision repair or double-strand break repair, can lead to substitutions, insertions, or deletions, thus creating oncogenes or inactivating tumor suppressor genes, such as P53. Viral infections, especially with viruses like human papillomavirus (HPV), can also play a major role in the initiation of cancer. HPV can express proteins, such as E6 and E7, that impair normal cell cycle controls by binding to proteins, disrupting the regulation of cell cycle progression and thus threatening genomic integrity. In addition, environmental factors, such as excessive exposure to sunlight (UV radiation), result in pyrimidine dimers, especially between thymine

bases, which compromise the integrity of regulatory genes and promote the accumulation of genomic changes.

6.3 Promotion phase (Epigenetics)

Cancer promotion is usually associated with epigenetic mechanisms such as DNA methylation in gene promoter regions, which can inhibit the expression of tumor suppressor genes, thus promoting cell proliferation and histone modifications by post-translational labeling on histones, such as acetylation or phosphorylation, influencing chromatin structure, making some genes more or less accessible for transcription. For example, histone acetylation is often linked to gene activation, while methylation can be associated with gene repression. Many agents, such as certain hormones or chronic irritation caused by physical or chemical substances, can serve as promoters. Significant promoters in humans include alcohol, foreign bodies (such as asbestos), as well as various infections and inflammations. Irritation can be mechanical (e.g., gallbladder stones), chemical (e.g., alcohol), or related to viral and bacterial infections. Other factors, such as radiation (UV rays, X-rays), nutritional elements (nitrosamines, high-fat diets), occupational factors (benzene, asbestos), and even certain drugs (arsenic derivatives, amphetamines) can act as promoters.

6.4 Progression phase (genetics and epigenetics)

During this third phase, precancerous lesions (such as polyps, dyskeratoses, or papillomas) continue to evolve even without promotion because their proliferation becomes autonomous. Mutagenic agents, whether endogenous or exogenous and epigenetic events promote the emergence of new features associated with invasive cancers in certain cells already presenting precancerous lesions. It is possible to track the evolution of these precancerous lesions in areas such as the skin, oral cavity, cervix, or colonic or gastric mucosa. Although cancer often originates from these precancerous lesions, it is important to note that only 1% of these lesions progress to cancer, as the majority regress spontaneously. The acquisition of uncontrolled functions, such as independence, loss of differentiation, and initiation of local and metastatic invasion, marks this phase.

6.5 Angiogenèse et metastase

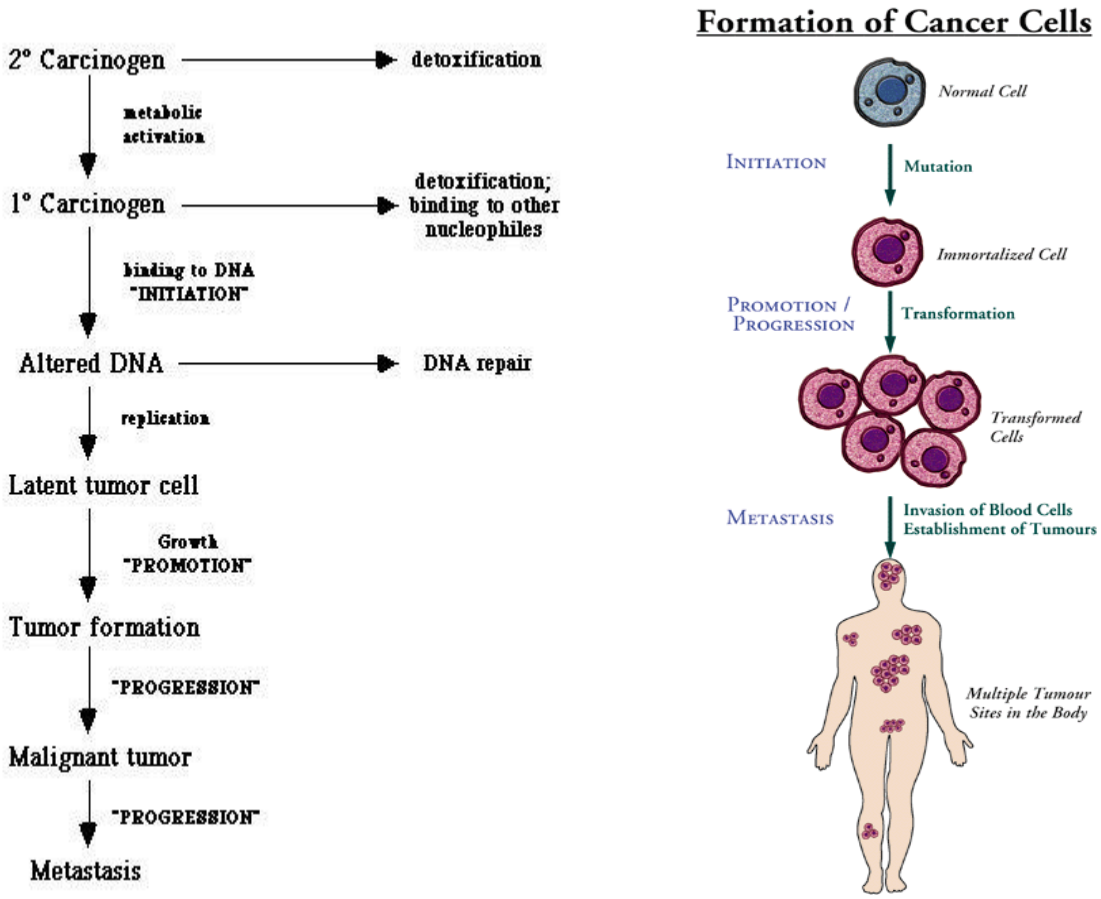


Figure 47: formation of cancer cells

6.6 The different clinical stages of cancer (TNM)

In terms of clinical classification, cancer is often described using the TNM system:

- T (Tumor): refers to the primary tumor size.
- N (Lymph nodes): indicates if the lymph nodes are affected.
- M (Metastases): refers to the presence or absence of metastases.

Based on the TNM system, general stages have been established:

- Stage 0 (carcinoma *in situ*): at this stage, the cancer is early and is confined to its original site without spreading.
- Stage I (localized): The cancer is small and usually localized in a single organ without lymph node involvement, but it may have invaded nearby tissues.

- Stage II: The cancer is larger and/or has spread nearby but remains localized.
- Stage III: the cancer is more advanced, with significant involvement of the lymph nodes, but without metastasis.
- Stage IV (metastatic): The cancer has spread to other parts of the body.

These classifications are very important because they make it possible to establish the appropriate treatment and to assess the chances of recovery and, therefore survival.

7 THE DIFFERENT STAGES AND MOLECULAR EVENTS OF TUMOR AND METASTATIC INVASION

7.1 Introduction

Tumor invasion and metastasis are complex processes that involve several molecular steps and events. Indeed, all cancers are likely to metastasize with varying frequencies and delays. The acquisition of metastatic potentialities by tumor cells requires phenotypic modifications concerning the interactions between the cells themselves or between the cells and the stromal microenvironment.

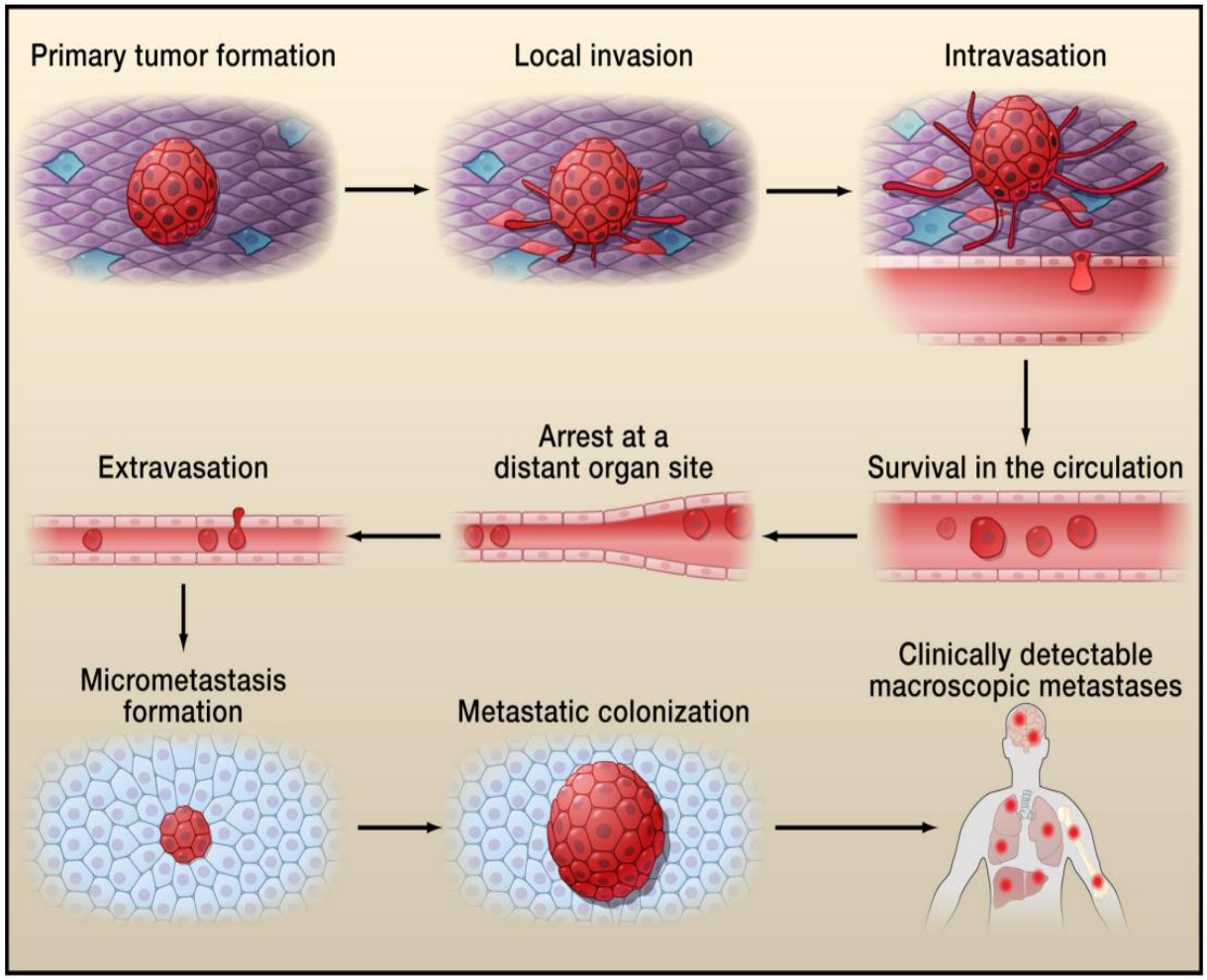


Figure 48: The different stages of the metastatic cascade (case of an epithelial tumor

In what follows, we will try to explain these molecular events to realize the extent of the complexity of tumor behavior. Here are the main molecular steps and mechanisms involved**:**

7.2 Detachment of tumor cells from the primary tumor

7.2.1 Loss of cell adhesion

Tumor invasion is characterized by the degradation of the basal matrix and the migration of cancer cells into the adjacent stroma. This process involves several key molecular mechanisms:

- **Modulation of adhesion molecules:** Tumor cells alter the expression of intercellular adhesion molecules, especially cadherins, and matrix adhesion molecules, such as integrins. This modulation leads to a reduction in cell cohesion.
- **Interactions with the extracellular matrix:** Interactions between cells and the extracellular matrix (ECM) are essential for biological processes such as proliferation, differentiation, and maintenance of tissue integrity. Adhesive molecules fall into two major classes:
- **Cell Adhesion Molecules (CAM):** They facilitate intercellular interactions. CAMs contain immunoglobulin motifs that ensure specific interactions between cell types. They interact closely with integrins.
- **Substrate Adhesion Molecules (SAM):** They participate in the binding between cells and ECM.

7.2.2 Intercellular adhesion

E-cadherin is a transmembrane glycoprotein essential for cell-to-cell adhesion in adherent junctions, connecting epithelial cells through its N-terminal extracellular domain and transmembrane region. It interacts with various adaptor proteins via its C-terminal cytoplasmic domain, playing a crucial role in maintaining tissue cohesion and resistance to shear forces, such as those encountered in the bloodstream.

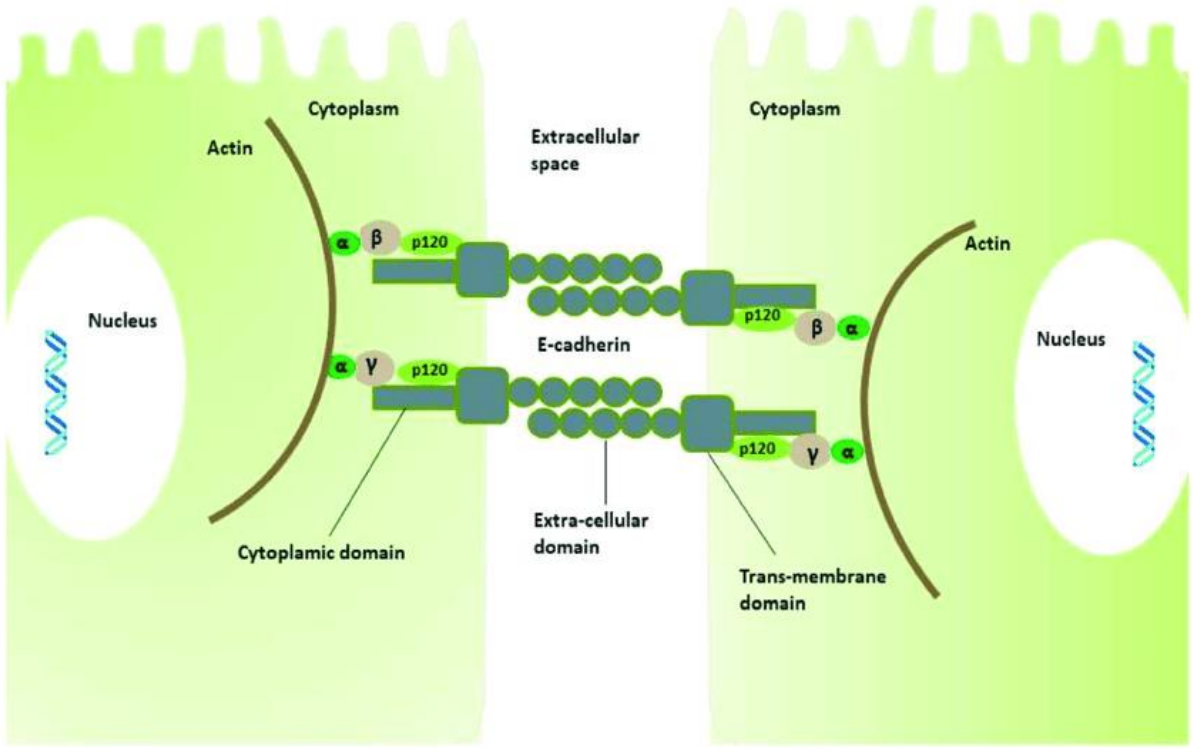


Figure 49: diagram of the role of E-cadherins and their structure

Loss of E-cadherin leads to decreased cohesion and increased invasiveness of cancer cells, contributing to metastatic phenotypes and an unfavorable prognosis in several types of cancers. This destabilization is often associated with an alteration of the cadherin-catenin complexes. For example, the APC (Adenomatous Polyposis Coli) protein, which regulates the degradation of β-catenin, becomes dysfunctional when it mutates or loses. This causes the accumulation of β-catenin, leading to the activation of oncogenic genes and promoting tumor progression.

In addition, the dysregulation of E-cadherin, due to mechanisms such as deletions, mutations or epigenetic modifications, represents a key step in tumor development. Hereditary Gastric Cancer Syndrome of the Diffuse Type (CGHD), for example, results from a mutation in the CDH1 gene, which encodes E-cadherin, predisposing to other cancers, such as invasive lobular breast cancer.

In parallel, immunoglobulins, such as N-CAM, I-CAM, and V-CAM, play a critical role in the interactions between immune cells and their partners, such as endothelial cells. These molecules, belonging to the immunoglobulin superfamily, have structured extracellular domains that promote specific interactions that are crucial for cell recognition and the regulation of immune responses.

E-cadherin and immunoglobulins are fundamental for maintaining cellular and tissue integrity. Their dysregulation has major consequences on tumor progression and metastasis, illustrating the importance of molecular interactions in cell and pathological biology.

7.2.3 Adhesion to the matrix

Integrins are heterodimeric membrane receptors composed of two subunits, alpha and beta, with two cytosolic domains and two extracellular domains. The alpha subunit binds to bivalent cations (Ca^{2+}, Mg^{2+}), and these units' extracellular ends interact with different components of the extracellular matrix, such as collagen, fibronectin, and laminin. A short sequence of amino acids recognizes fibronectin, while other integrins bind specific sequences of collagen and laminin.

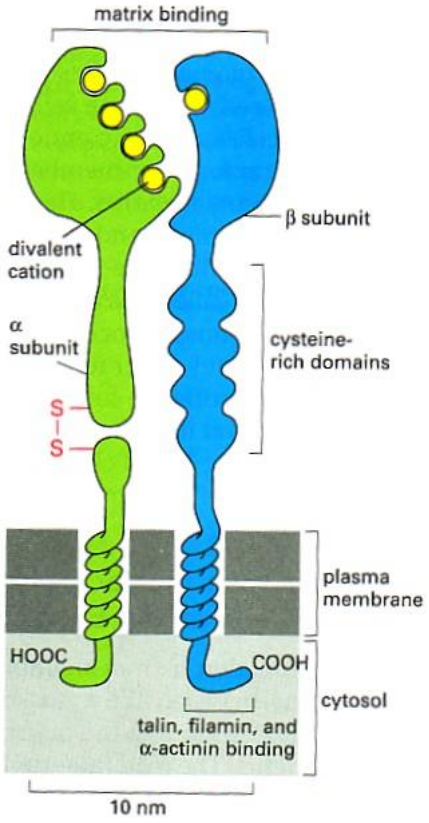


Figure 50: Schematic representation of an integrin

When an integrin attaches to a ligand, its beta-subunit recruits adaptor proteins like tensin and talin, connecting the integrin to actin filaments and thus establishing a link between the extracellular matrix and the cytoskeleton. This process is crucial for the formation of focal contact points, which are essential for cell migration.

Other signaling proteins, such as FAK (Focal Adhesion Kinase) and ILK (Interleukin-Linked Kinase), are also activated, impacting pathways related to cell survival, proliferation, and migration.

The β1, β4, and αv families of integrins bind to various elements of the matrix, while β2 is specific to leukocytes and plays a role in cell-to-cell adhesion. The expression and affinity of integrins vary in cancer cells. For example, αvβ3 integrin is highly expressed in malignant melanoma, and α6β4 is induced in thyroid carcinomas. Integrin α5β1 can decrease tumorigenesis in certain cell lines. Integrins are essential for cancer cell migration and survival; for example, activation of β1 integrin in lung cancer cells reduces the expression of ICAM-1. Integrin β4, often amplified in carcinomas (skin, breast, kidney, colon), promotes cell invasion and migration by relocating from hemidesmosomes to lamellipods. Finally, integrin can support the survival of p53-deficient cancer cells by activating the AKT/PKB pathway, which increases VEGF translation and stimulates tumor progression through an autocrine effect, as in breast carcinoma.

7.3 Selectins

Selectins play a key role in cell adhesion and metastatic dynamics of cancers, making them potential targets for developing therapeutics aimed at limiting tumor spread. Selectins are crucial for interacting with endothelial cells and other cells, such as lymphocytes (L-selectin) or platelets (P-selectin). Selectin E, in particular, is expressed by endothelial cells in response to cytokines like interleukin 1, interferon-gamma, and tumor necrosis factor. These molecules are the first to intervene in the inflammatory response and play an essential role in rolling polynuclear cells and tumor cells along the endothelium.

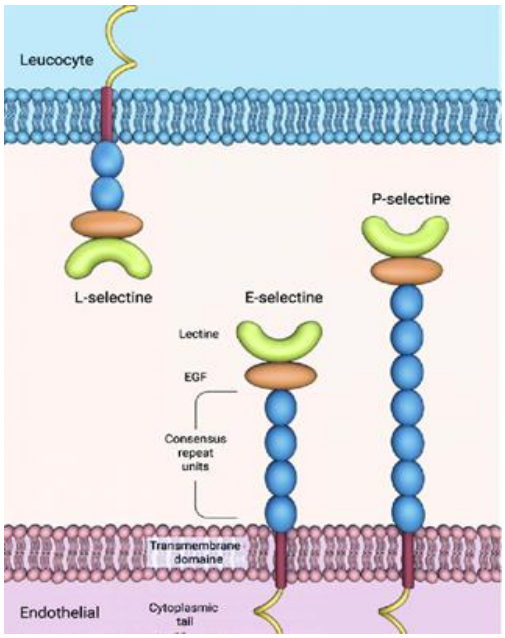


Figure 51: structure of the different selectins

Selectins allow circulating cell adhesion to the endothelium, acting as a "brake" on blood flow. However, a definitive adhesion of cancer cells or lymphocytes requires the participation of other molecules, such as integrins and CD44 molecules. Initially, circulating cells attach and roll along vascular endothelial cells through the interaction between E selectin and its ligand, allowing for rolling and adhesion. In the second step, called stopping, the cells adhere firmly to the endothelial cells, spread over the endothelium, and actively migrate through the endothelial wall. Chemokines play a critical role in this stopping step by activating integrins on leukocytes, thus facilitating robust adhesion via ICAM and VCAM molecules, allowing their transmigration across the endothelial wall.

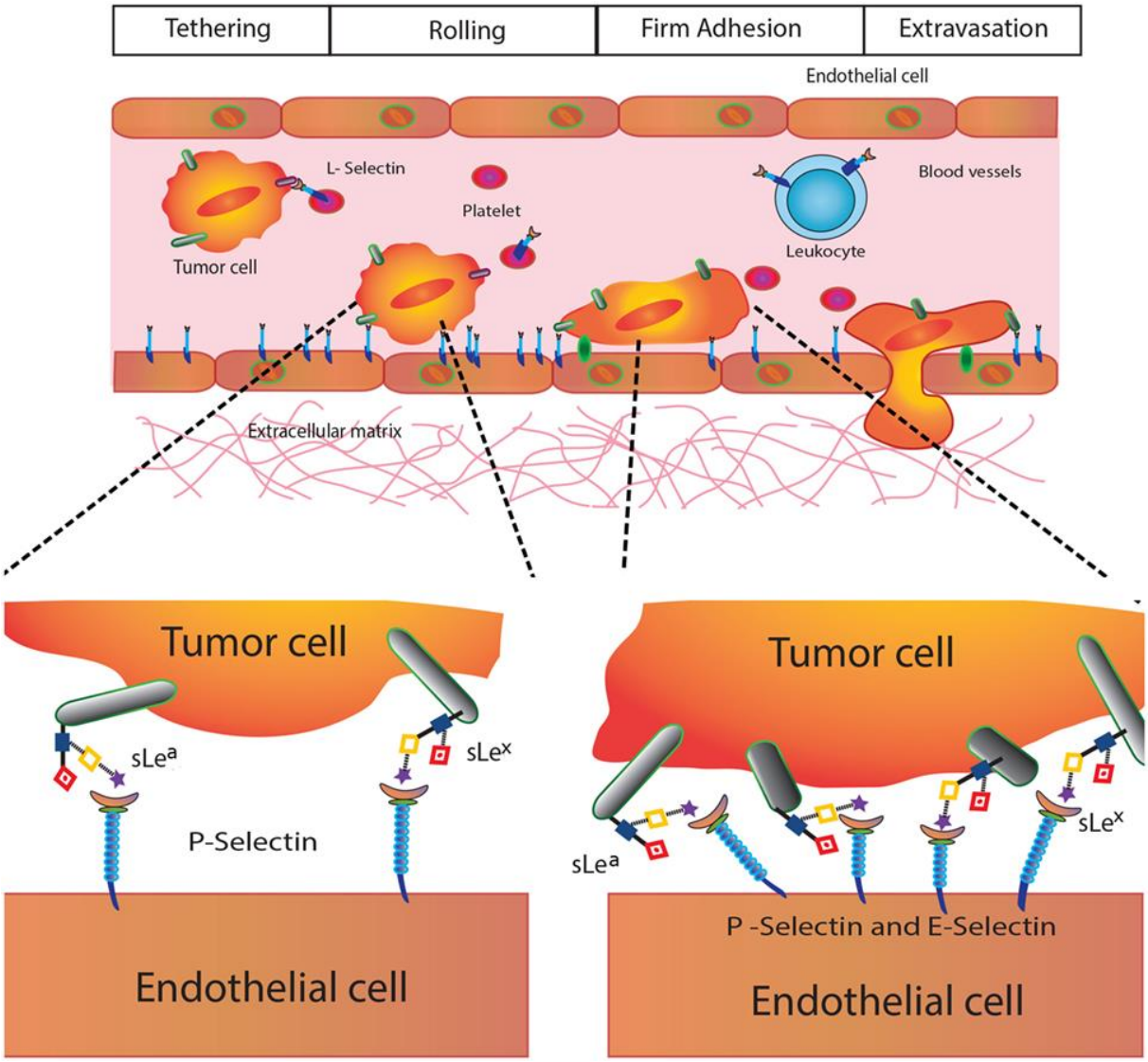


Figure 52: Cell Adhesion Mechanisms in Tumor Cell Extravasation: Role of Selectins

Each selectin shares a similar structure, endowed with a carbohydrate recognition domain (CRD) with an affinity for specific oligosaccharides bound to proteins or lipids. In particular, binding to E selectin is crucial for the adhesion of epithelial cancer cells to the endothelium. The more a cancer cell can bind to E selectin, the higher its metastatic potential.

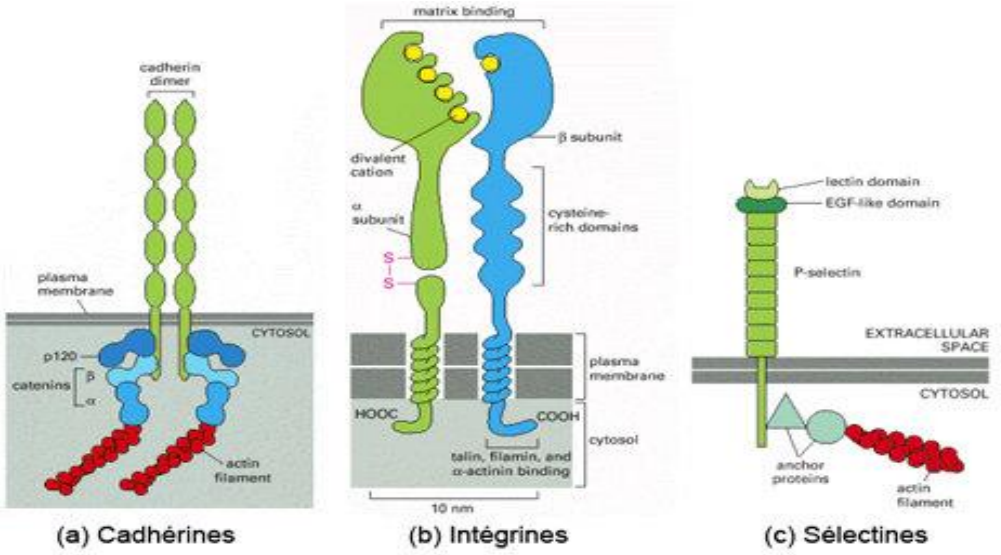
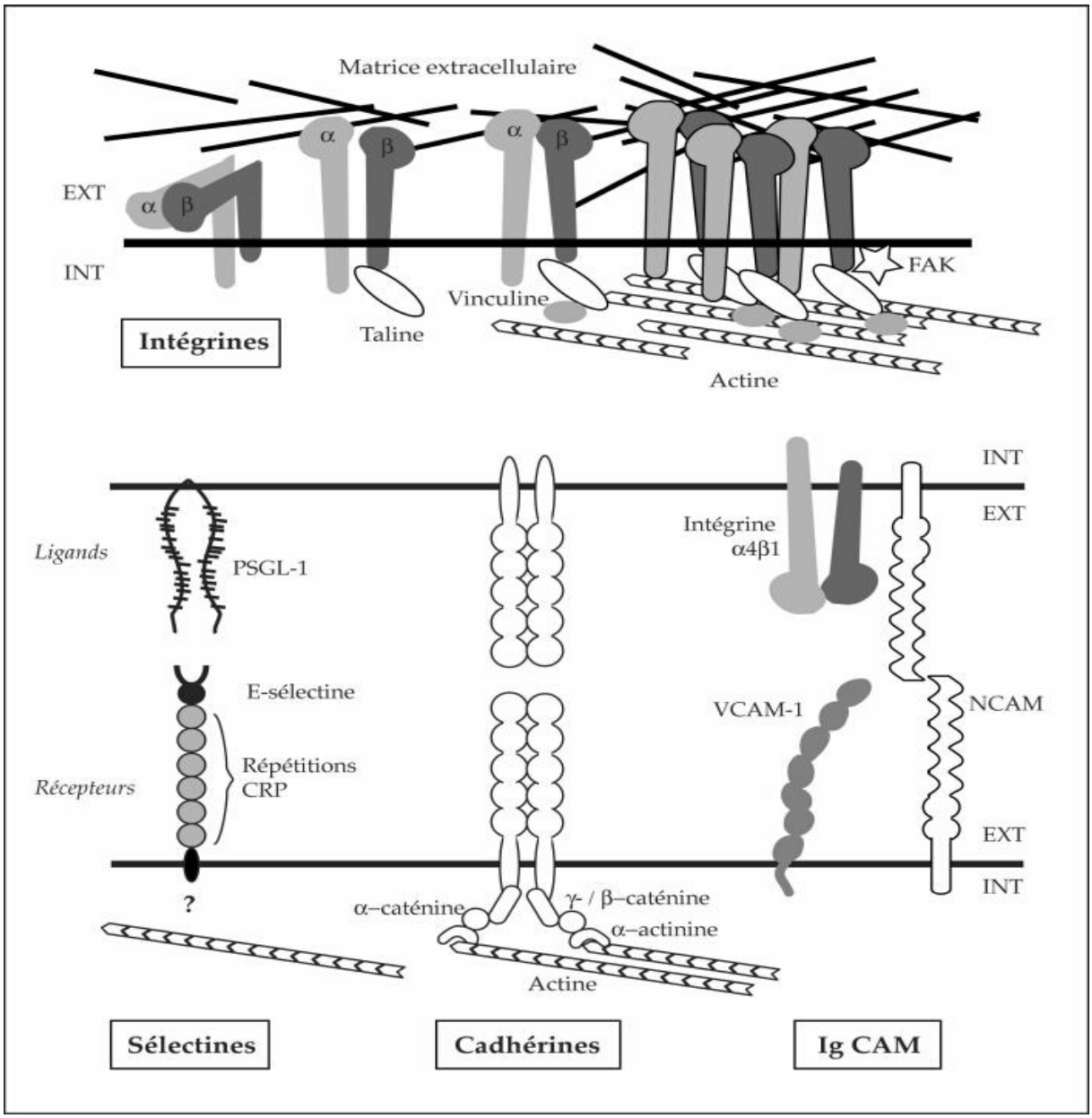


Figure 53: Diagrams of the three main families of adhesion molecules

Studies show that cimetidine, a drug that inhibits the expression of E selectin independently of its anti-histamine effect, can reduce tumor cell adhesion and inhibit the formation of metastases. In addition, the use of antisense targeting E selectin in the liver showed reduced liver metastases from colorectal carcinomas. Another antisense acting on fucosyltransferase, which generates the ligand of selectin E in cancer cells, was also effective against these metastases. Introducing a soluble recombinant form of Selectin E into lung metastasis models also attenuated cancer cell adhesion and decreased metastasis formation. Finally, the expression of E selectin on Lewis's lung carcinoma cells' sinusoidal endothelium of the liver appears to increase their metastatic potential, which could explain hepatic colonization. Clinically, circulating selectin E levels are correlated with metastasis, especially in breast cancer patients; elevated concentrations of advanced blood-soluble selectin E are associated with liver metastases. On the other hand, low levels of this soluble selectin are favorable prognostic indicators for survival and recurrence in patients with node-negative breast cancer.



Figures 54: the four main families of adhesion molecules

7.3.1 CD44s

Surface and transmembrane molecules, such as CD44, are crucial in various physiological pathways. They promote adhesion between cells and extracellular matrix (ECM) elements, such as fibronectin, collagen, and hyaluronic acid. CD44 is often expressed by cancer cells, playing a role in migration, homing, and metastasis growth. Through cleavages by metalloproteinases (MMPs), CD44 can enter the nucleus and act as a transcriptional activator, thus regulating cell motility and ECM renewal. Its binding with collagen IV and other components of the basement membranes also facilitates tumor invasion. CD44 expression varies according to tumors,

making it possible to differentiate between forms with low metastasizing and those with high metastatic potential, particularly in lung, colon, and breast cancers.

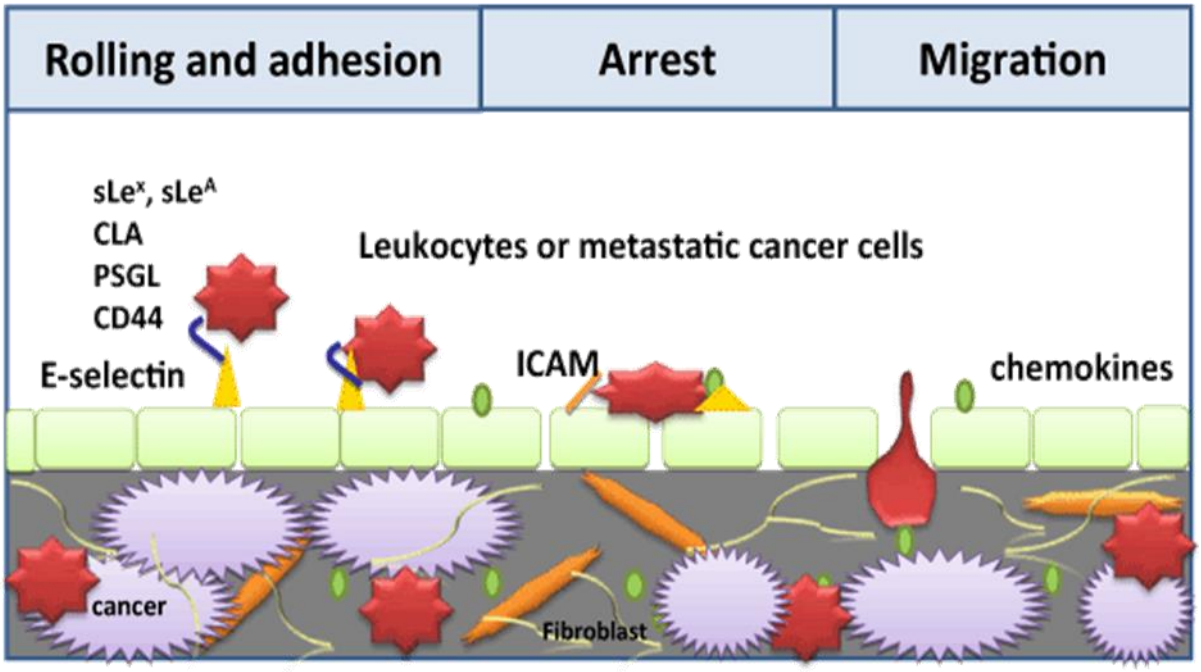


Figure 55: Adhesion cascade for leukocytes and circulatory metastatic cancer cells.

7.4 Degradation of the extracellular matrix

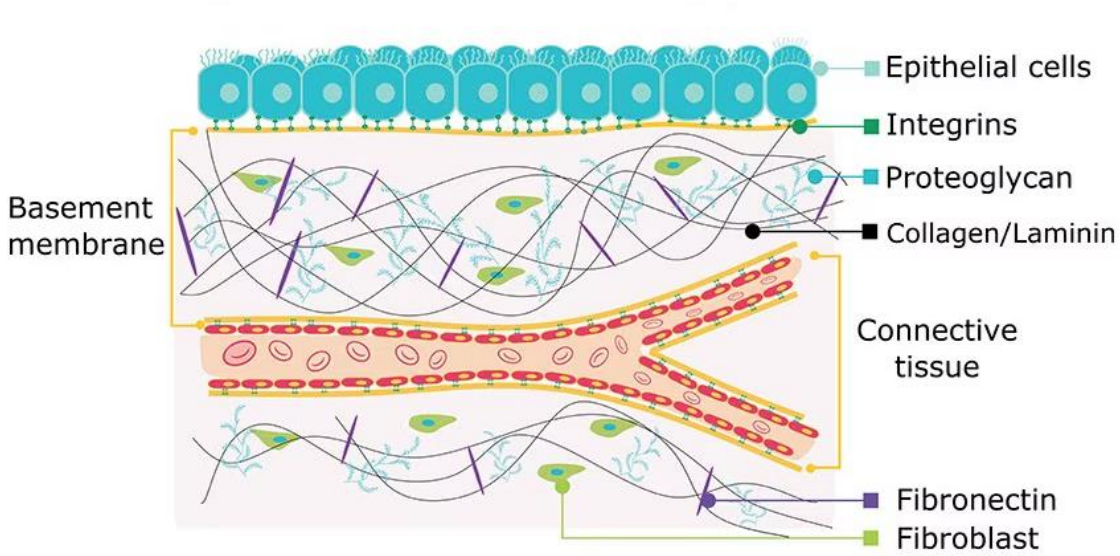


Figure 56: composition of the extracellular matrix

Proteases:

Cancer cells can degrade the constituents of the basement membrane and extracellular matrix (ECM), a process crucial for tumor progression. This proteolysis involves enzymes secreted by cancer cells but also by stromal cells, in particular fibroblasts, which are stimulated by diffusible factors emitted by tumor cells. Three main enzymatic activities have been identified in this context: the uPA/uPAR (pro-urokinase/pro-urokinase receptor) system, metalloproteinases, and cathepsin B.

uPA/uPAR system: Pro-urokinase (uPA) is a plasminogen-activating serine protease, present in an inactive form before being cleaved by plasmin. This cleavage allows uPA to initiate a proteolytic cascade, leading to the remodeling and degradation of the ECM and basement membrane. When uPA binds to its uPAR receptor on the cell surface, it concentrates its proteolytic activity there, facilitating tumor invasion.

Matrix metalloproteinases (MMPs): This broad family of endopeptidases can degrade a wide range of ECM proteins, with 25 human genes identified and pseudogenes. MMPs are organized into four subfamilies, classified according to their substrate specificity (collagenases, stromelysin, gelatinases, etc.), and will target collagen I and VI, proteoglycans, lamins, and fibronectin. As soon as they are synthesized, MMPs are inactive (pro-enzyme), requiring cleavage to become active, often facilitated by membrane-bound MMPs (MT-MMPs). Their activity is regulated by tissue inhibitors such as metalloproteinase inhibitors (TIMPs). Notably, TIMP-1 can reduce metastasis formation in animal models, while TIMP-2 inhibits ECM invasion.

Cathepsin B: As the cysteine protease of lysosomes, cathepsin B exists as procathepsin B, which is cleaved and glycosylated to become active. Its activation involves a complex entanglement with other enzymes, including uPA, elastase, and other cathepsins (D and G). In turn, cathepsin B contributes to the activation of plasminogen-plasmin cascades and the uPA/uPAR system and may also indirectly activate certain MMPs.

Thus, the degradation of ECM by these enzymes constitutes a key mechanism in tumor progression, facilitating the invasion and dissemination of cancer cells. This understanding of the molecular mechanisms involved opens up prospects for new therapeutic strategies aimed at inhibiting these degradation pathways and slowing cancer progression

7.5 Cell motility and migration:

Cell motility refers to the ability of cells to perform spontaneous or reactive movements, and it is crucial in many physiological processes, such as embryogenesis, wound healing, angiogenesis, and inflammation, as well as in pathological contexts, such as tumor growth and metastasis. After inhibition of the different pathways involving extracellular matrix (ECM) proteolytic cascades, tumor cells have been shown to retain significant migratory capacity. These cells appear to make their way through the ECM by mobilizing their cytoskeleton rather than clearing their path through proteolytic action.

The cytoskeleton, which determines the cell's shape, comprises three main elements: actin microfilaments, intermediate filaments, and microtubules. The actin microfilament array is significant for cell movements due to its polymerization and depolymerization properties and its contractile capacity in interaction with myosin. During migration, two protrusions are generated: lamellipods and filopods, which involve different molecular mechanisms and factors.

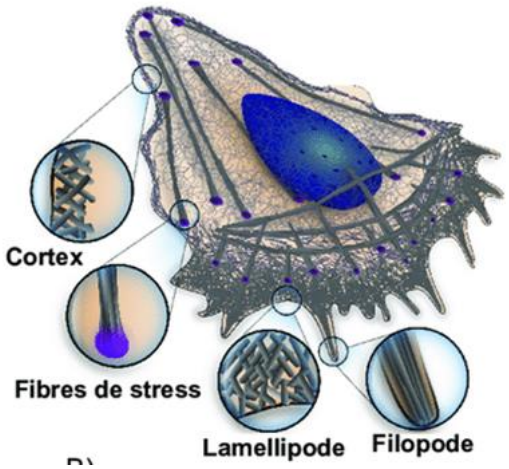


Figure 57: Structural Components of a Migrating Cell: Cortex, Stress Fibers, Lamellipodia, and Filopodia

Cell migration is a multi-step process, finely regulated:

- **-Membrane protrusion**: The cell becomes polarized, and cortical actin polymerizes to project the membrane forward, forming the filopods and initiating signaling.
- **-Formation of adhesion focal points**: Integrins interact with the ECM, recognizing ligands and oligomerizing to recruit adhesion plate proteins, kinases, and actin-binding proteins, which creates a cytoskeleton anchor point.

- **-Protease recruitment**: ECM interaction sites stimulate the recruitment of proteases such as matrix metalloproteinases (MMPs), which cleave the surrounding matrix, exposing new sites and freeing up space for migration.
- **-Cell contraction**: Myosin generates a contraction force on the polymerized actin fibers, allowing the back of the cell to move forward.
- **-Detachment from the back of the cell:** The focal points of adhesion at the rear unravel, recycling the components towards the front of the cell.

These steps are schematized in the following figure

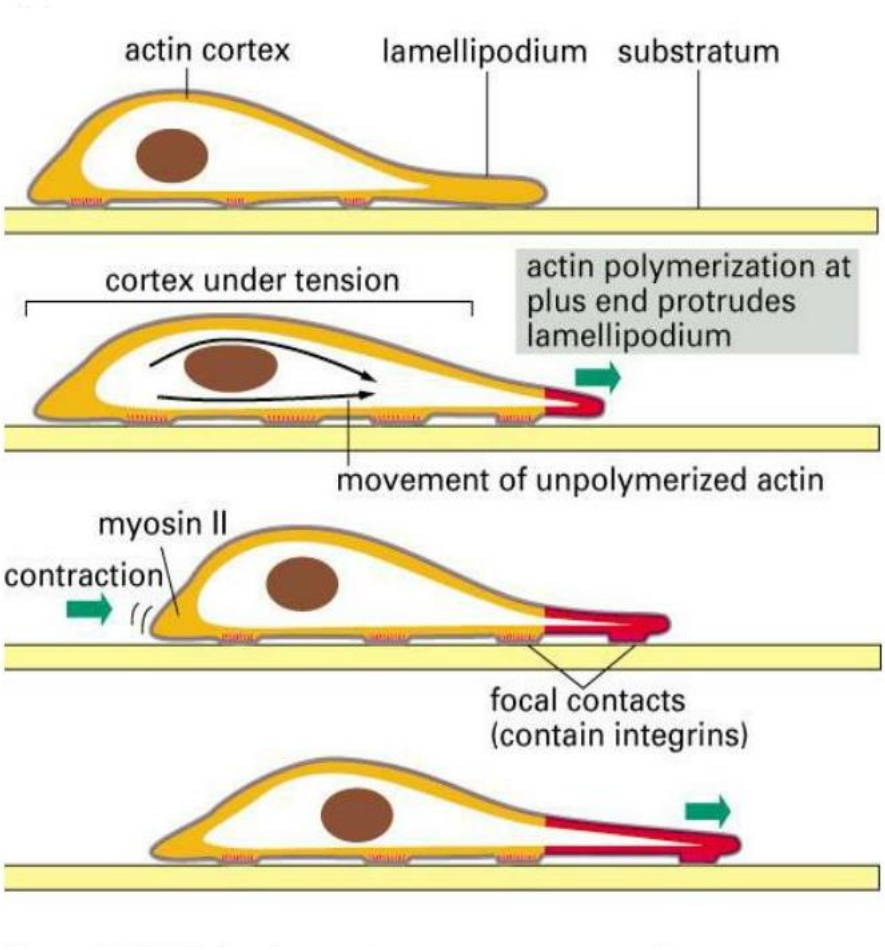


Figure 58: Different stages of cancer cell migration.

Focal adhesion kinase (FAK) plays a central role in this process, being activated by integrins, growth factors, and hormones. Invalidation of the FAK gene in cells leads to a significant reduction in their ability to migrate in vitro, highlighting the importance of this kinase in the regulation of cell motility.

One of the particularities of cancer cell migration is their plasticity. Indeed, these cells can adapt to a new environment or constraints by modifying their migration type. A key aspect of this plasticity is the epithelial-mesenchymal transition (EMT). Cancer cells exploit the epithelial-mesenchymal transition (EMT), a process that temporarily transforms epithelial cells into mesenchymal cells, allowing them to migrate. Once they arrive at their site of invasion, these cells undergo a mesenchymal-epithelial transition (MET), transforming them back into epithelial cells to rebuild the damaged epithelium. This natural process of cell transformation gives cells the ability to migrate and reach the mesenchyme. Among the triggering signals for EMT, transforming growth factor beta (TGF-β) plays a crucial role. It is produced by macrophages and fibroblasts within the tumor but can also be synthesized by the cancer cells themselves. EMT is characterized by the loss of epithelial properties, such as polarity and cell junctions, in favor of mesenchymal traits that promote motility. This change allows cells to migrate more efficiently to invasion sites and interact differently with the extracellular matrix

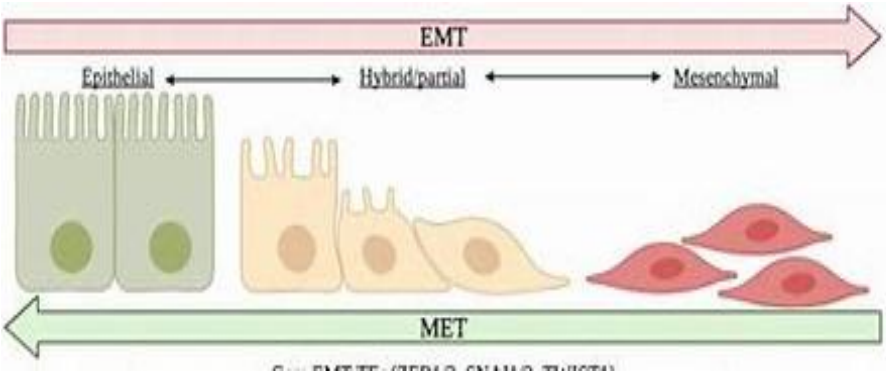


Figure 59: EMT and MET transition

EMT transition is often associated with increased metastatic potential and has been shown to be regulated by a variety of factors, such as growth factors, cytokines (TGF-β (transforming growth factor-beta), and extracellular matrix modifications.

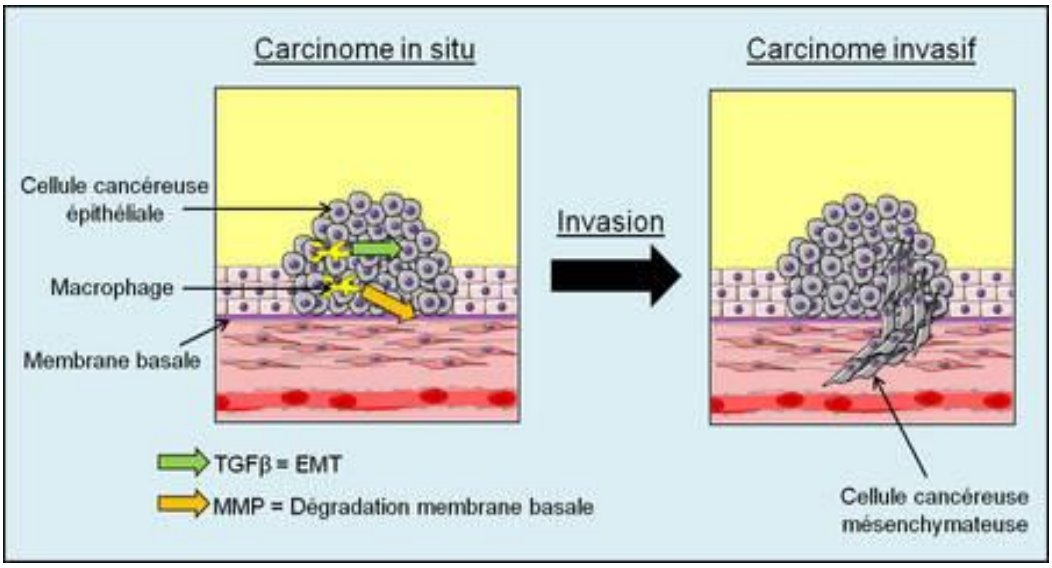


Figure 60: schematic representation of carcinome *in situ* and invasif

The plasticity of the migration mechanisms is illustrated in the figure. In addition to the migration of isolated cells, a distinction is made between collective migration, where a group of cells or a sheet uses common mechanisms to invade tissue, without requiring the dissemination of individual cells (Figure).

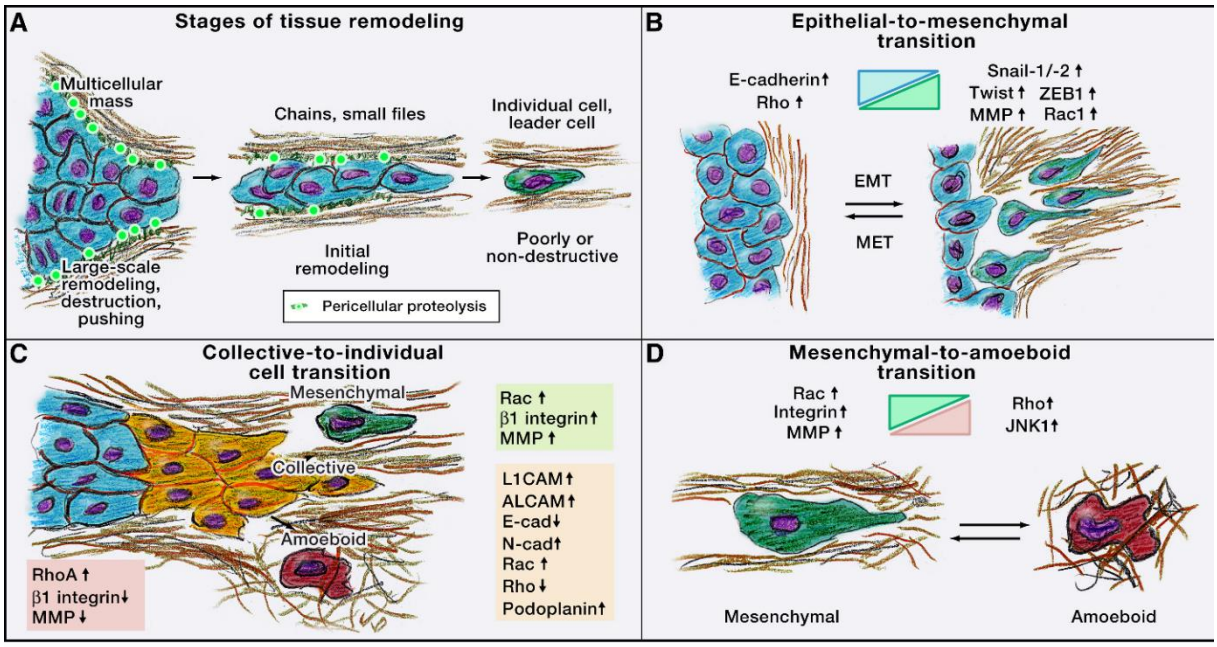


Figure 61: Transitions Between Mesenchymal and Amoeboid Cell Phenotypes: Key Molecular Changes

To adapt to their microenvironment, tumor cells are able to adopt different modes of migration, which are mainly distinguished by their morphology. In a sparsely populated environment, cells

migrate using an amoeboid mode, characterized by the formation of blebs or short protrusions. On the other hand, in a denser environment, they adopt a mesenchymal mode of migration. When multiple cells follow a "leader" cell, it can form a multicellular flow, which can evolve into collective migration if the junctions between the cells are maintained. Cells that migrate amoeboid have a round shape, while those that adopt a mesenchymal mode have an elongated shape. During collective migration, cells are organized into two groups: the "guides", located at the front of the group, which exert tensile forces on the fibers to move, and the "followers", which, as their name suggests, follow the guides.

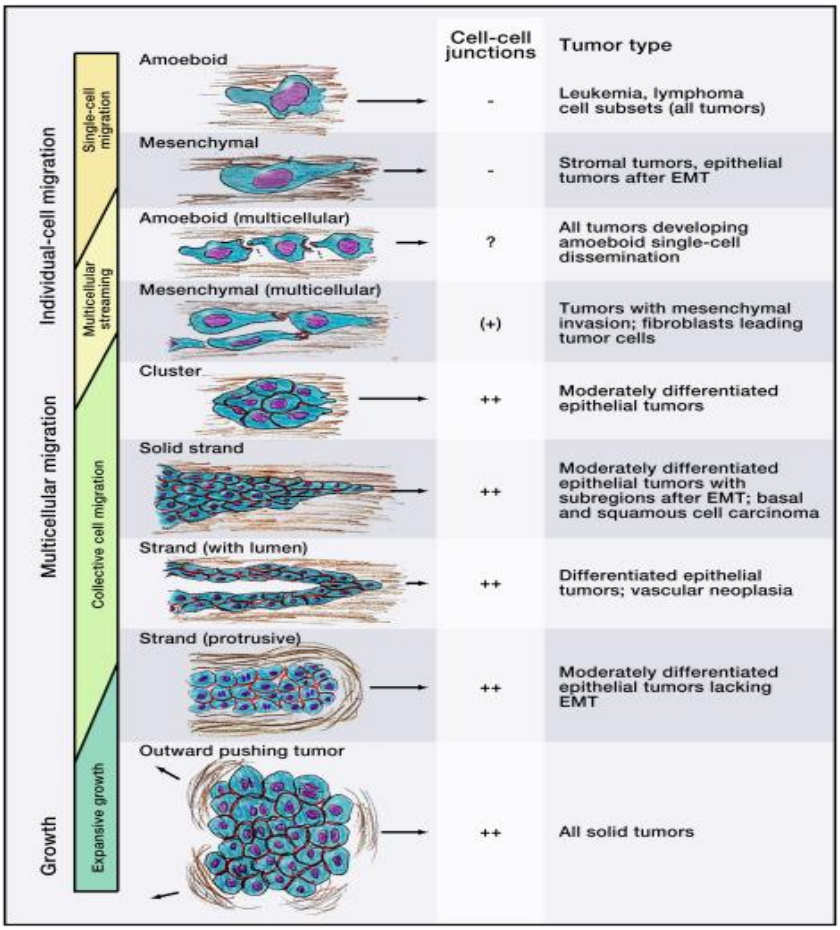


Figure 62: The different modes of cell migration.

This ability to adapt to changes in environmental conditions underlines the importance of understanding these processes for developing effective therapeutic strategies against cancer.

As the tumour grows and grows, it reaches a point where the supply of oxygen and nutrients provided by the pre-existing blood vessels becomes insufficient. At this stage, the cancer cells trigger angiogenesis and induce the formation of new blood vessels. This process is crucial for ensuring tumour survival and progression, allowing the tumour to continue to grow.

7.6 Angiogenesis

Angiogenesis, defined as the process of forming new blood vessels from pre-existing vessels, plays a fundamental role in tumor biology. As tumor cells grow and proliferate, they quickly reach a size where oxygen and nutrients supplied by the existing blood vessels become insufficient. To overcome this limitation, tumors develop angiogenesis capabilities, releasing growth factors such as vascular endothelial growth factor (VEGF), which stimulate neovascularization.

This ability to induce angiogenesis is not only a mechanism for tumor growth; It is also associated with cancer progression, metastatic dissemination, and resistance to treatments. As a result, angiogenesis has become a major therapeutic target in developing new cancer treatments. Understanding the underlying mechanisms of angiogenesis allows us to not only shed Light on cancer disease processes but also to explore new therapeutic strategies to improve patient outcomes. In this part of our course, we will take a detailed look at the mechanisms of angiogenesis, its crucial role in cancer, and the therapeutic approaches that result from it.

7.6.1 Tumor Angiogenesis at the Target Organ Level

To grow, tumor cells need access to nutrients, gas exchange and eliminate waste products generated by their intense metabolism. To meet these needs, they trigger the formation of new blood vessels, a process called neo-angiogenesis. Angiogenesis is a biological mechanism that allows the formation of new vessels from existing blood vessels. This process begins with specific signals that recruit and stimulate the proliferation of endothelial cells, which come together to form the wall of the new capillaries. It starts with forming a cell bud that then opens to form a tube through which blood can flow, connecting to other capillaries.

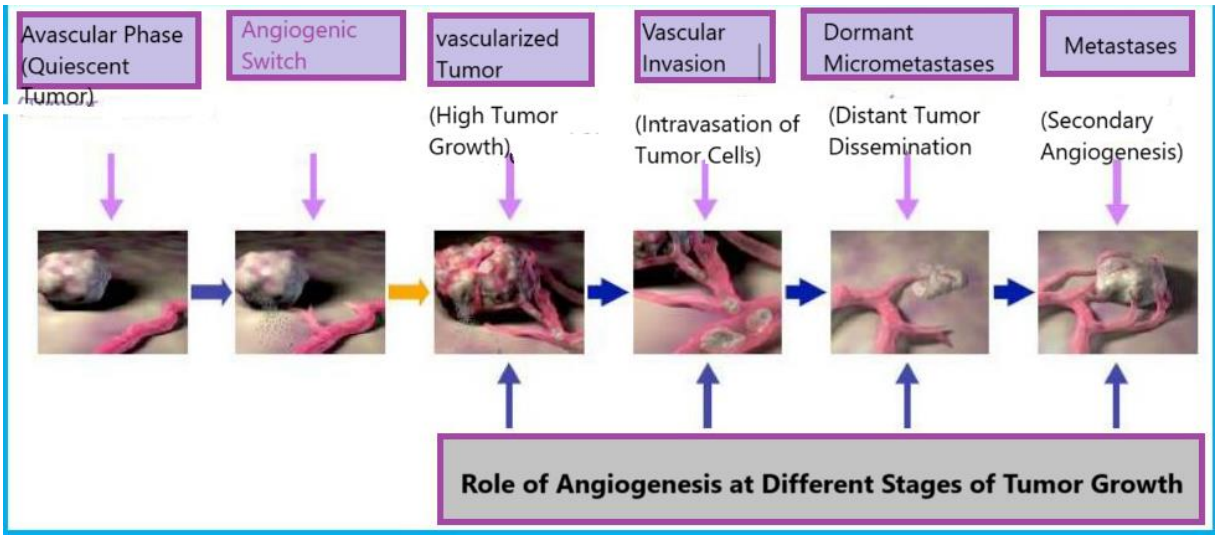
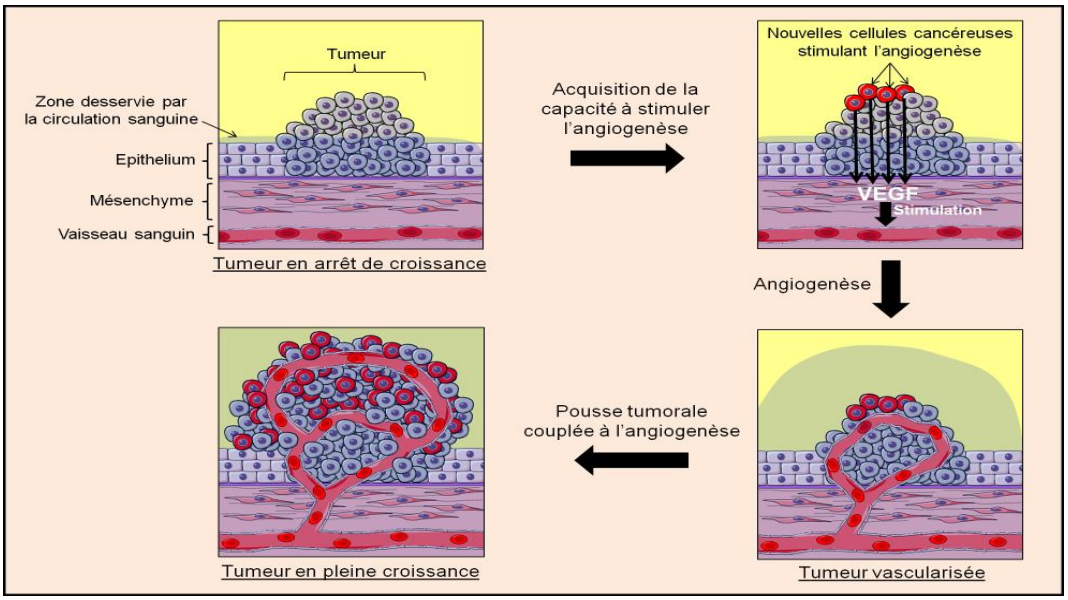


Figure 63: the role of angiogenesis in the different stages of tumor growth

Key steps in this process include:

1. Degradation of the basement membrane of capillaries, leading to angiogenic stimulation.
2. The migration of endothelial cells across the basement membrane.
3. The need to maintain the stimulus, because if it is removed, the neo-capillaries begin to regress and eventually disappear. Thus, a metastatic nodule deprived of its vascularization stops growing when its size reaches about one to two mm^3.



Figures 64 : tumor angiogenesis

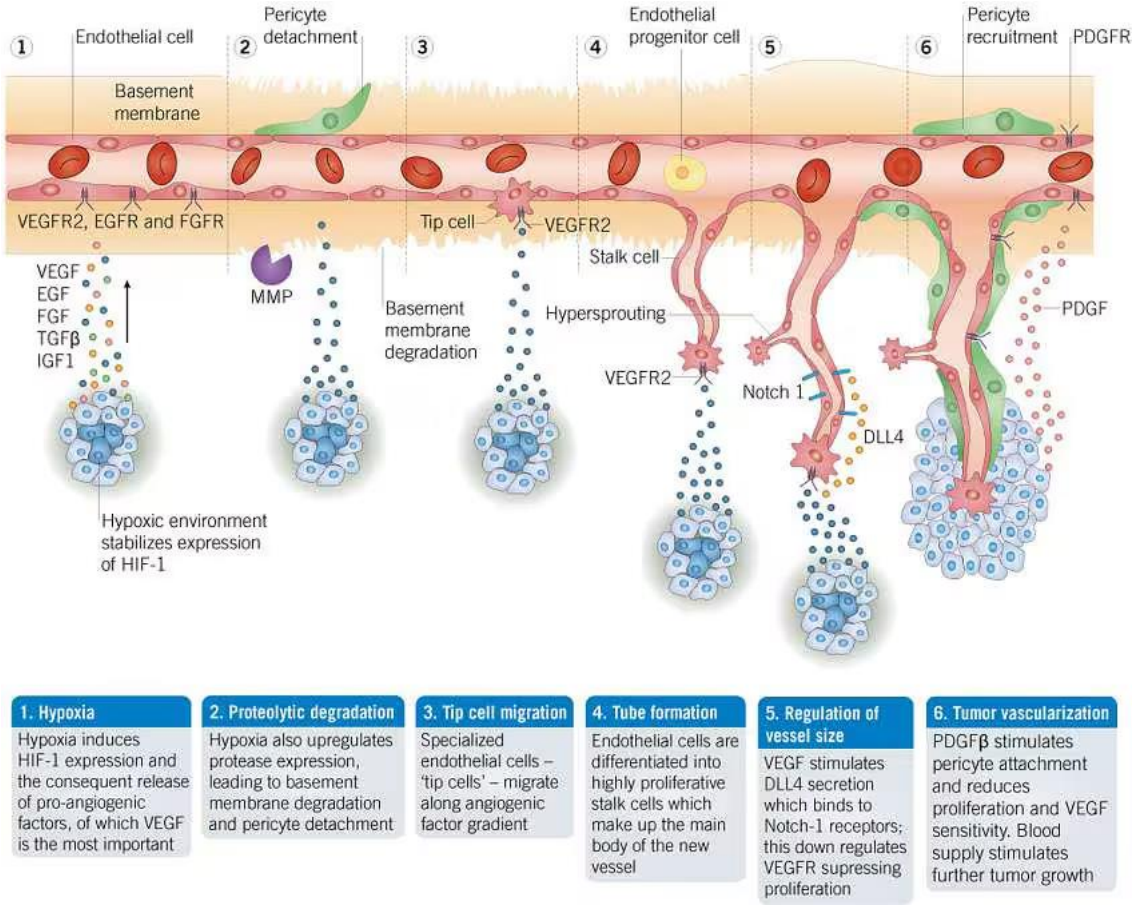


Figure 65: Different stages of tumor angiogenesis

7.6.2 Malignant cells induce angiogenesis by two main pathways

7.6.2.1 Direct pathway

In this case, cancer cells directly activate endothelial cells. Many factors, including cytokines such as EGF, FGF-1, FGF-2, FGF-3, FGF-4, PDGF (platelet-derived growth factor), TGF (transforming growth factor), and VEGF (vascular endothelial growth factor), have been identified for their angiogenic properties. VEGF is considered to be the main player in this process, its production is induced by a lack of oxygen within the cells, which then secrete it. VEGF diffuses into tissues and acts on neighboring endothelial cells via receptors, prompting them to migrate and proliferate toward the source of the signal.

7.6.2.2 Indirect pathway

This pathway involves the secretion of angiogenic factors by tumor cells, which in turn act on cells in the microenvironment. For example, immune cells, fibroblasts, and stromal cells can be recruited and activated by these factors. Tumor-associated macrophages, in particular, release

cytokines and other mediators that stimulate the release of VEGF and other pro-angiogenic factors.

In addition, cell-level activation of the Notch pathway in response to ligands like DLL4 also plays a crucial role, influencing the behavior of endothelial cells and promoting the stabilization of new vessels. This interaction helps regulate vascular development, allowing tumor cells to direct the formation of new vessels in response to their needs.

When new vessels form and bring blood to the tissue, the oxygen concentration increases, leading to a decrease in VEGF production and an interruption of angiogenesis. This mechanism is crucial during tumor growth, especially when the oxygen concentration within the tumor mass becomes insufficient. Thus, angiogenesis is regulated by VEGF levels, the interaction between DLL4 and Notch, and cell-derived signals from the tumor microenvironment.

When tumor cells reach the blood vessels, they must pass through the vessel wall to enter the circulation. This intravasation process requires active migration and degradation of the extracellular matrix of the vascular epithelium, often mediated by the same signaling pathways that regulate EMT.

7.7 Intravasation

Intravasation is a key process in the formation of metastases in cancer. Intravasation is the process by which tumor cells enter the bloodstream or lymphatic from the primary tumor. This usually occurs after tumor cells have successfully broken through tissue barriers and begin to invade blood vessels. This phenomenon is essential for the formation of metastases and is regulated by multiple molecular and cellular factors.

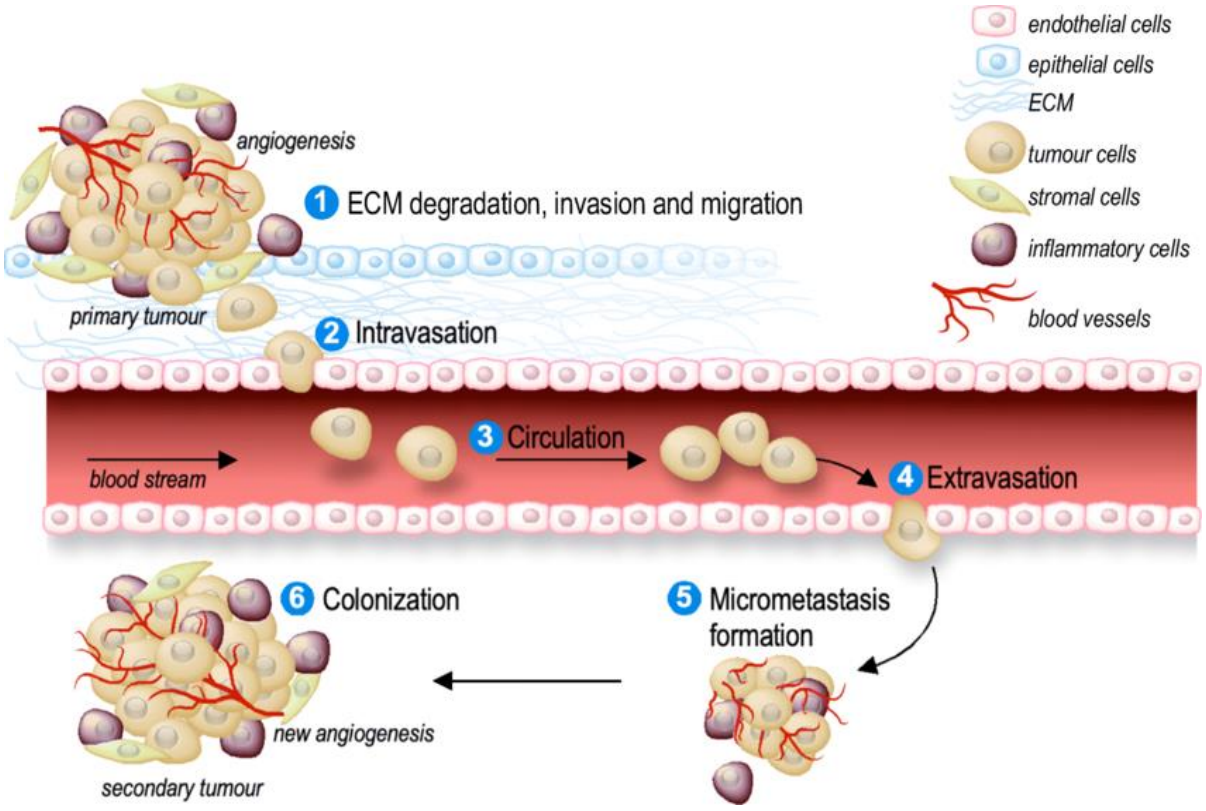


Figure 66: the different step of intravasation

After intravasation, cancer cells enter the vascular system, which is a key step in the metastasis process. Once in the bloodstream or lymphatic, they face several challenges before reaching a new organ to form metastases. First, circulating tumor cells (CTCs) must survive in a hostile environment. The immune system, the mechanical forces of blood flow, and oxidative stress represent major obstacles. Some cancer cells manage to avoid these threats by associating with platelets, forming cellular aggregates that temporarily protect them.

7.8 Immune system escape

Tissue monitoring by the immune system consists of the detection and elimination of cells infected by pathogenic or cancerous organisms.

The anti-tumor immune response is a complex process in which the immune system recognizes and destroys cancer cells. It is divided into two main phases: innate immunity and adaptive immunity.

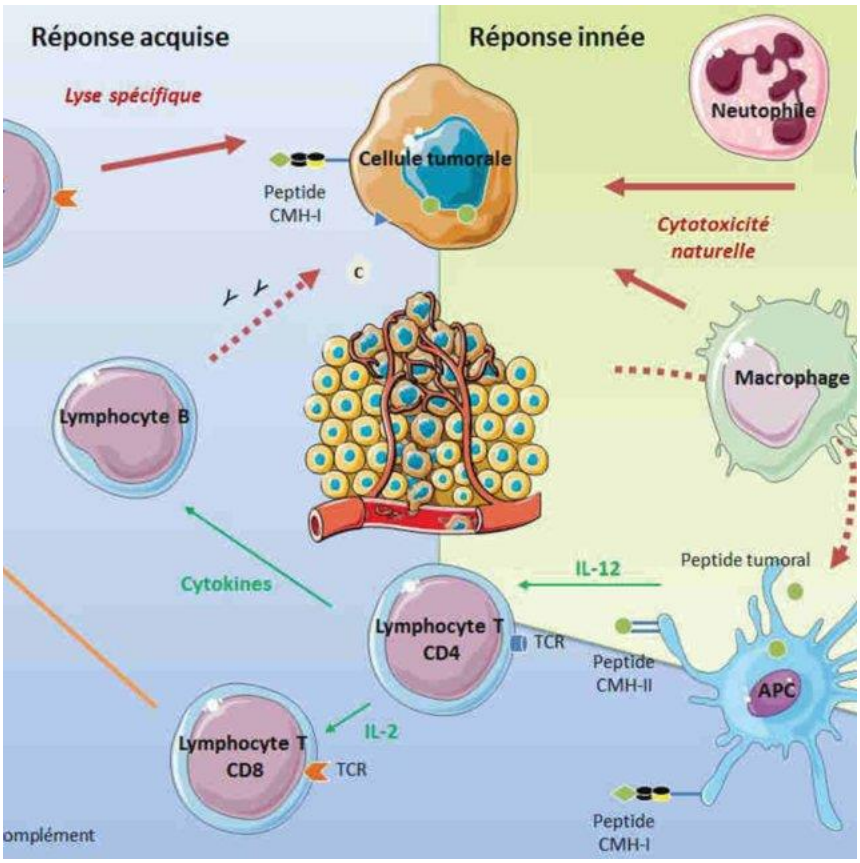


Figure 67: the interaction between the innate immune response and the aquired immune response in the context of tumor immunity

The immune establishment of cancer can be summarized by the so-called three E's: elimination, balance and escapement.

The concept of immunosurveillance comes into play during the elimination phase, where the immune system will allow the tumor to be destroyed. During this phase,

Various actors of the immune system will be recruited at the tumor site in order to limit its development. After the immune system eliminates the majority of tumor cells, a dynamic equilibrium phase is set up between the immune system and the surviving tumor variants. This phase can be seen as a period of Darwinian selection, during which many rapid genetic mutations will take place that will lead to the development of a clone resistant to the attacks of the immune system. This period, considered to be the longest of the three, can be spread over several years. During the last phase, the tumor variant(s) that have acquired insensitivity to detection and/or elimination by the immune system, begin their phase of uncontrolled growth. This then leads to the development of cancer. The immune system can be broadly divided into two innate and adaptive responses:

7.8.1 Innate immunity phase

This first line of defense is provided by cells such as macrophages, NK (natural killer) cells and dendritic cells (DC). These cells recognize tumor cells through specific molecular patterns on the surface of tumors, called PAMPs (Pathogen-Associated Molecular Patterns), via PRR (Pattern Recognition Receptors). When dendritic cells detect these signals, they secrete cytokines such as IFN-α, which activate other cells of innate immunity, thus increasing the immune response against the tumor.

The figure illustrates the key steps in the immune system's activation process against tumours, including the interaction between immune cells and cancer cells. The following is a detailed description of the components and steps depicted:

1. **Presentation of Cancer Antigens**: Dendritic cells (DCs) capture antigens from tumor cells and migrate to lymph nodes to activate T cells.
2. **T Cell Activation**: In the lymph nodes, DCs present antigens to T cells, resulting in their activation and initiating a targeted immune response.
3. **Amplification of the Innate Immune Response**: Macrophages, NK cells, and DCs are activated through tumor antigen signals, strengthening the immune response.
4. **Infiltration of T lymphocytes into the Tumor**: Activated T cells circulate in the blood and make their way to the tumor, guided by chemotactic signals.
5. **Tumor Recognition and Detection**: Tumor cells release antigens and DAMPs, signaling the presence of abnormal cells and stimulating the immune response.
6. **Destruction of Cancer Cells**: In the tumor, T cells recognize and kill cancer cells, while other immune cells such as macrophages also contribute to this destruction by phagocytosis.

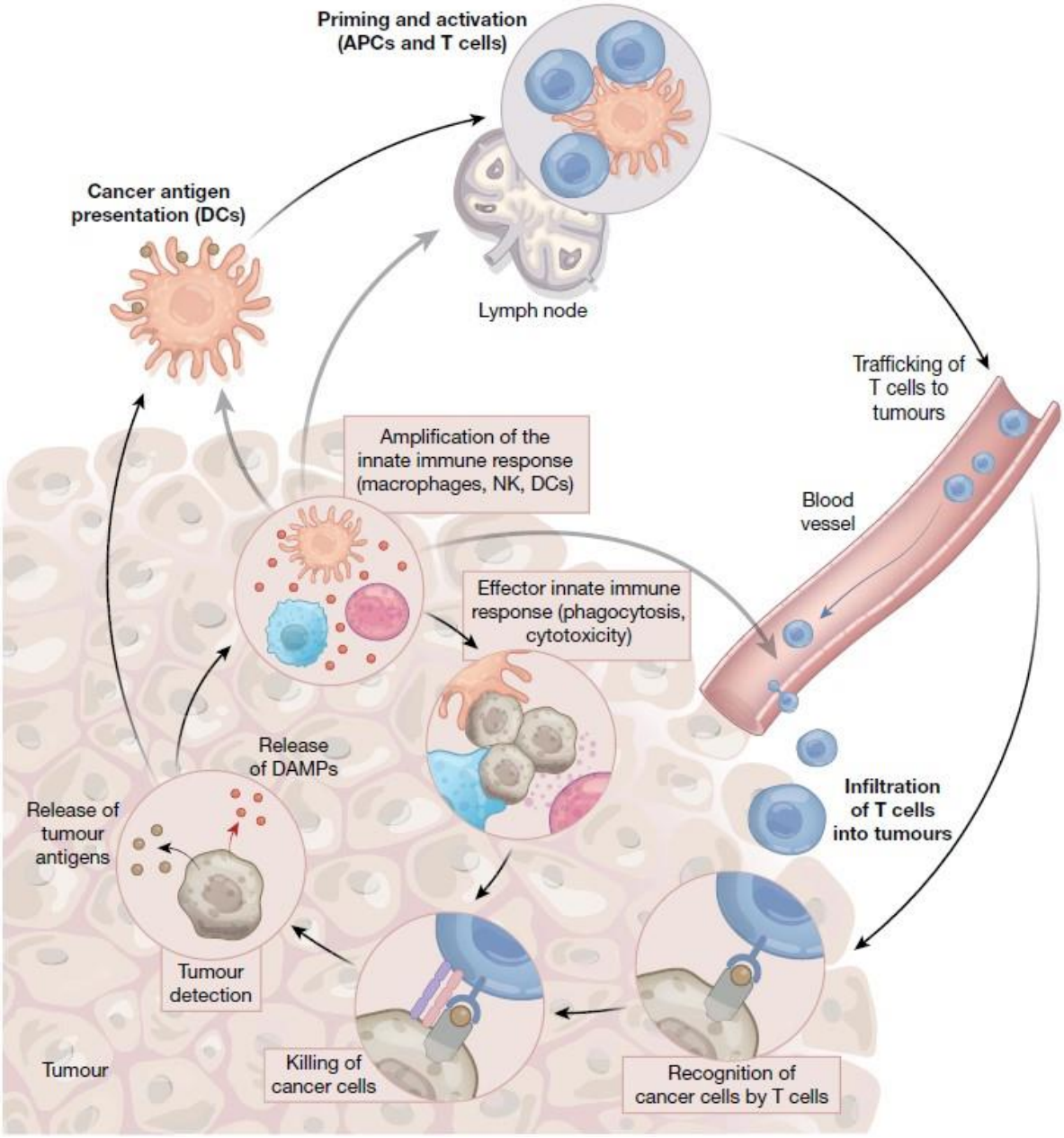


Figure 68: The diagram highlights the complex collaboration between the innate and adaptive immune systems in recognizing and eliminating tumor cells.

7.8.2 Adaptive immunity phase:

Antigens, fragments of molecules from the cell, are exposed to the immune system via the major histocompatibility complex (MHC). Effector lymphocytes, equipped with a TCR receptor, distinguish between self-antigens (from our body) and non-self-antigens (foreign). When they detect cancer cells, they activate and eliminate them. Cancer cells display antigens on their surface via MHC-I, which allows cytotoxic T cells to recognize and destroy them.

Dendritic cells (DCs), after capturing tumor antigens, present them to T lymphocytes in the lymph nodes. This activates cytotoxic T cells (CD8+), which specifically recognize cancer cells by binding to antigens presented by MHC-I. This activation leads to the destruction of cancer cells by apoptosis (programmed cell death)

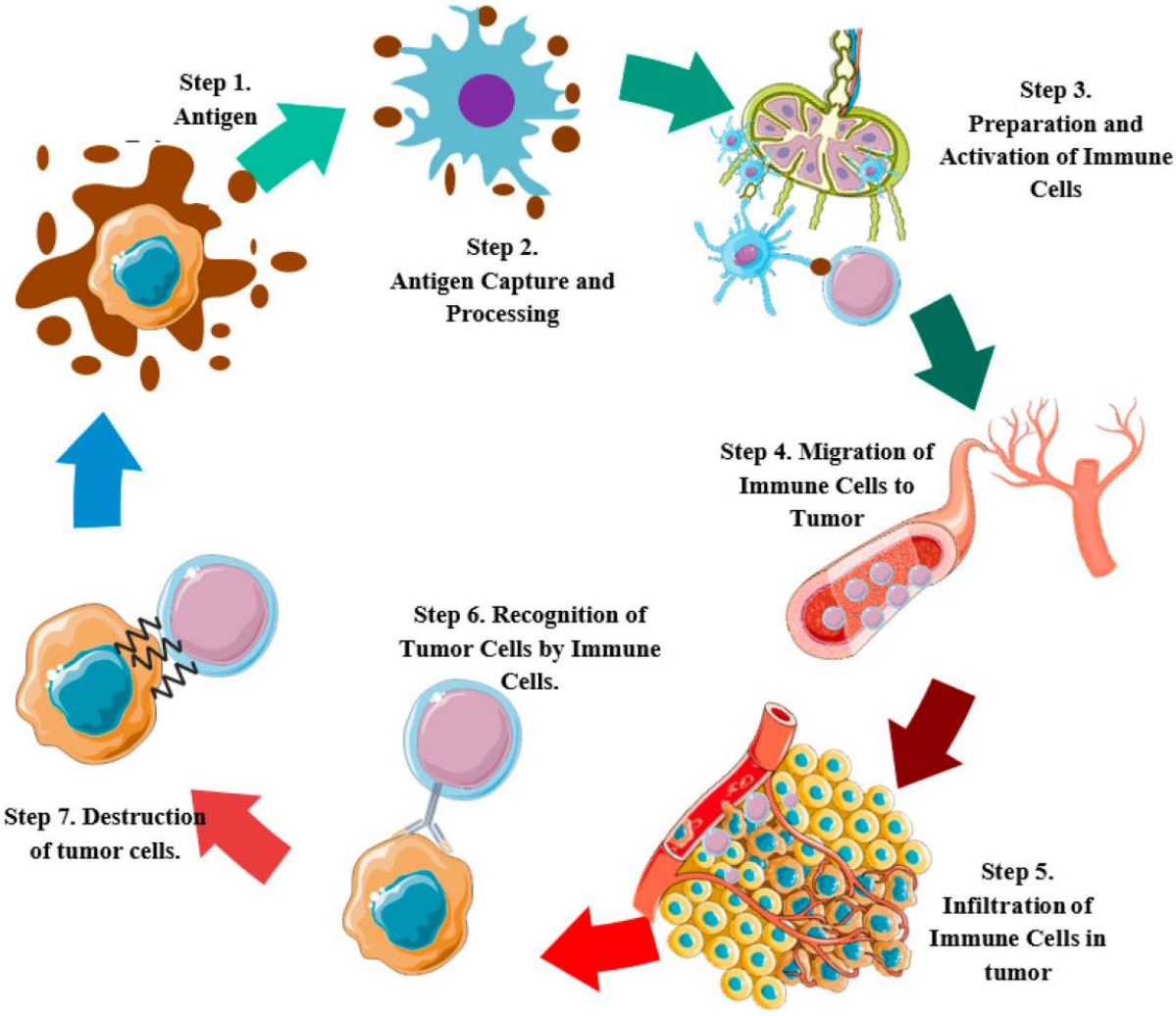


Figure 69: the step of the immune response in cancer

7.9 Regulation of the immune response

In addition to cytotoxic T cells, helper T cells (CD4+) play a supporting role by secreting cytokines to stimulate and maintain the antitumor immune response. However, regulatory cells, such as regulatory T cells (Tregs), can also modulate this response to prevent excessive destruction of normal tissue.

7.9.1 Escape of cancer cells from the immune response:

Tumor cells use several molecular mechanisms to evade the immune response and avoid recognition and elimination by the immune system. Here are some of the main mechanisms involved:

7.9.1.1 Escape by loss of MHC-I

The loss or alteration of the expression of major histocompatibility complex (MHC) molecules, often referred to as MHC (or HLA in humans), is an important mechanism by which tumor cells can evade the immune system. Tumor cells can lose or change the expression of tumor antigens, making it more difficult for T cells to recognize them. Cancer cells reduce or eliminate the expression of MHC-I, for example, by mutation or loss of specific genes, making antigenic presentation ineffective and tumor cells "invisible" to T cells.

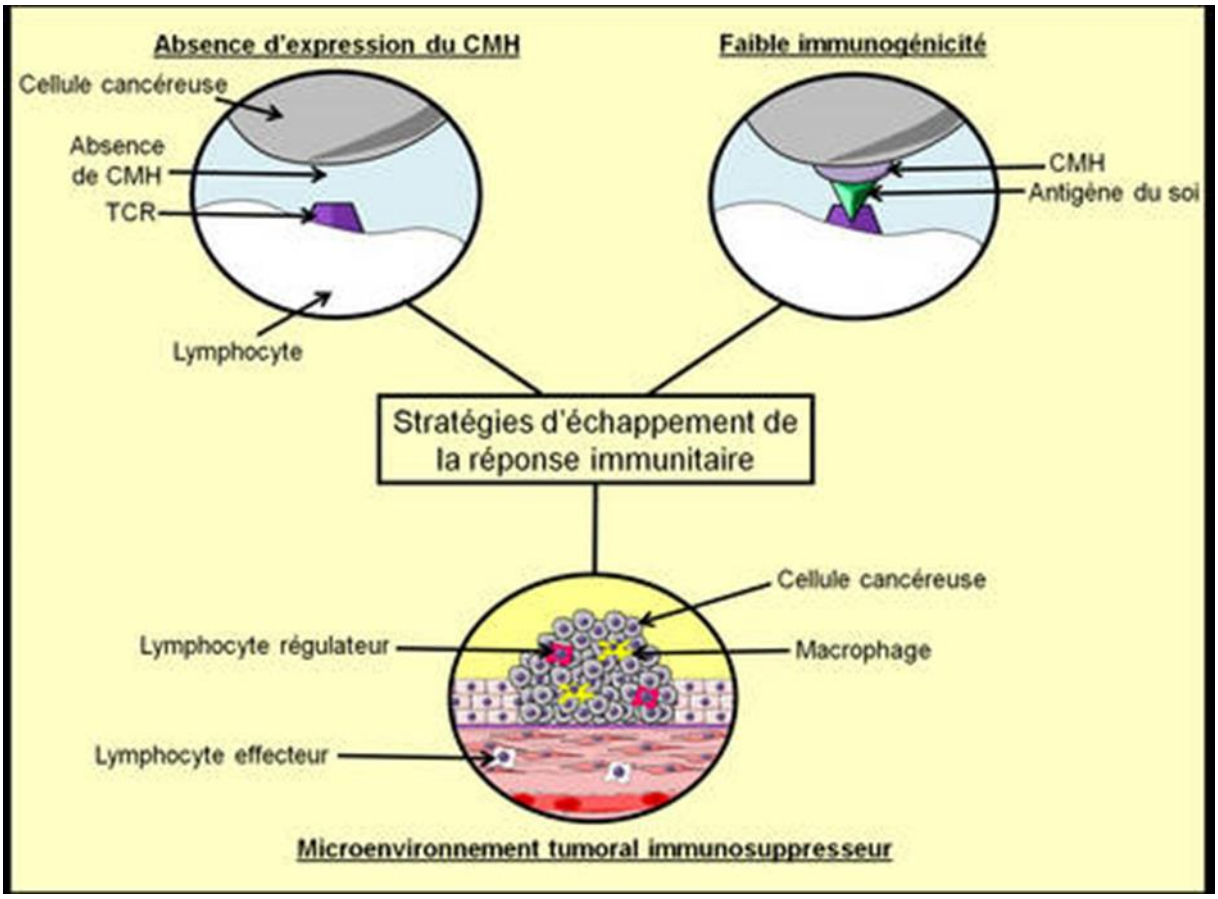


Figure 70: Escape of cancer cells from the immune response by loss of HLA

This can also be done via mutations in the TAP1 and 2 genes, responsible for the transport of antigenic peptides to the endoplasmic reticulum, leading to a degradation of MHC-I molecules, thus blocking the presentation of antigens to immune cells. Finally, epigenetic modifications, such as hypermethylation of the promoters of the MHC-I or TAP genes, prevent their expression, reinforcing the escape of cancer cells from immune surveillance.

7.9.1.2 Creation of an immunosuppressive microenvironment

The immunosuppressive environment within tumors plays a crucial role in bypassing the immune response, allowing tumor cells to grow without being attacked. This environment is characterized by the recruitment of immunosuppressive cells such as regulatory T cells (Tregs) and tumor-associated macrophages (TAMs), which inhibit effector T cells. Tumors also secrete cytokines like TGF-β, IL-10, and IL-6, which negatively modulate immune cell activity. In addition, some tumors can alter the expression of B7 molecules on antigen-presenting cells (APCs), reducing their ability to provide the co-stimulation needed to activate T cells. This decrease in B7 expression may result from genetic modifications, exposure to immunosuppressive cytokines produced by the tumor microenvironment, or interaction with

other immune cells. Antigen-presenting cells, such as macrophages and dendritic cells, can acquire an immunosuppressive phenotype, further compromising their ability to activate T cells. In addition, hypoxia in tumors alters cell metabolism, enhancing immunosuppression and disrupting immune cell function. Activation of inhibitory signaling pathways, such as PD-1/PD-L1, also limits the efficiency of T cells. These combined mechanisms make tumor treatment more complex and have led to the development of immunological therapies aimed at restoring the immune response.

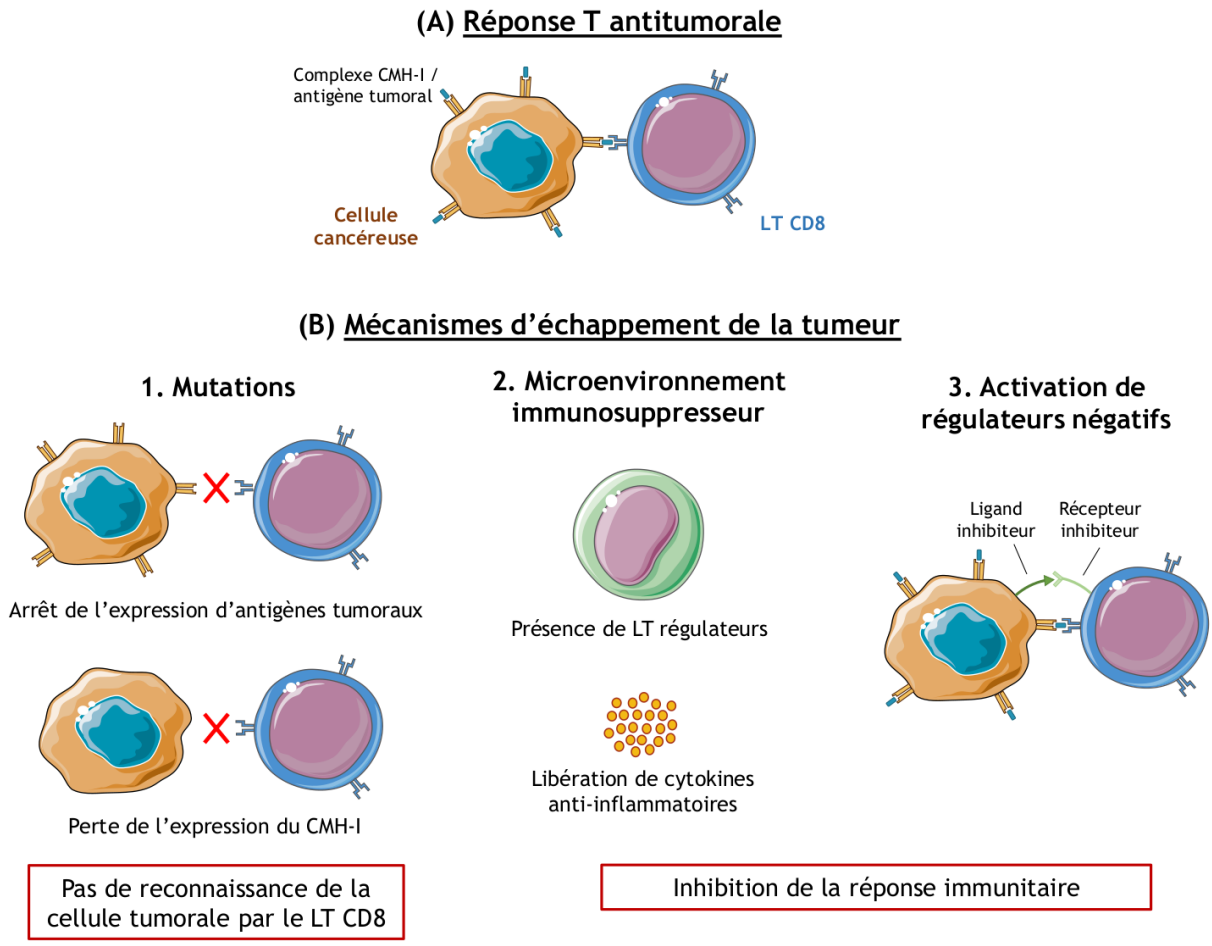


Figure 71: Mechanisms of tumor escape from the immune system. (A) During the immunosurveillance phase of the tumor, an antitumor T response is established following the recognition of the tumor cell by CD8 T cells. At the molecular level, this recognition involves the interaction between the tumor antigens presented by the MHC I of tumor cells and the TCR of T lymphocytes. These cells are then able to eliminate cancer cells thanks to their effector functions. (B) During the escape phase, different mechanisms limit the immune response: 1. Tumor cells undergo mutations, which can lead to a loss of expression of tumor antigens or MHC I molecules. The cancer cells can then no longer be recognized by CD8 T cells. 2. Within

the tumor, a suppressor microenvironment is set up, composed in particular of regulatory T lymphocytes and anti-inflammatory cytokines such as IL-10. These cells and molecules inhibit the immune response. 3. Tumor cells express inhibitory ligands (e.g., PD-L1) on their surface, which can bind to inhibitory receptors (e.g., PD-1) expressed by activated CD8 T cells. The activity of the latter is then inhibited, and these cells die by apoptosis.

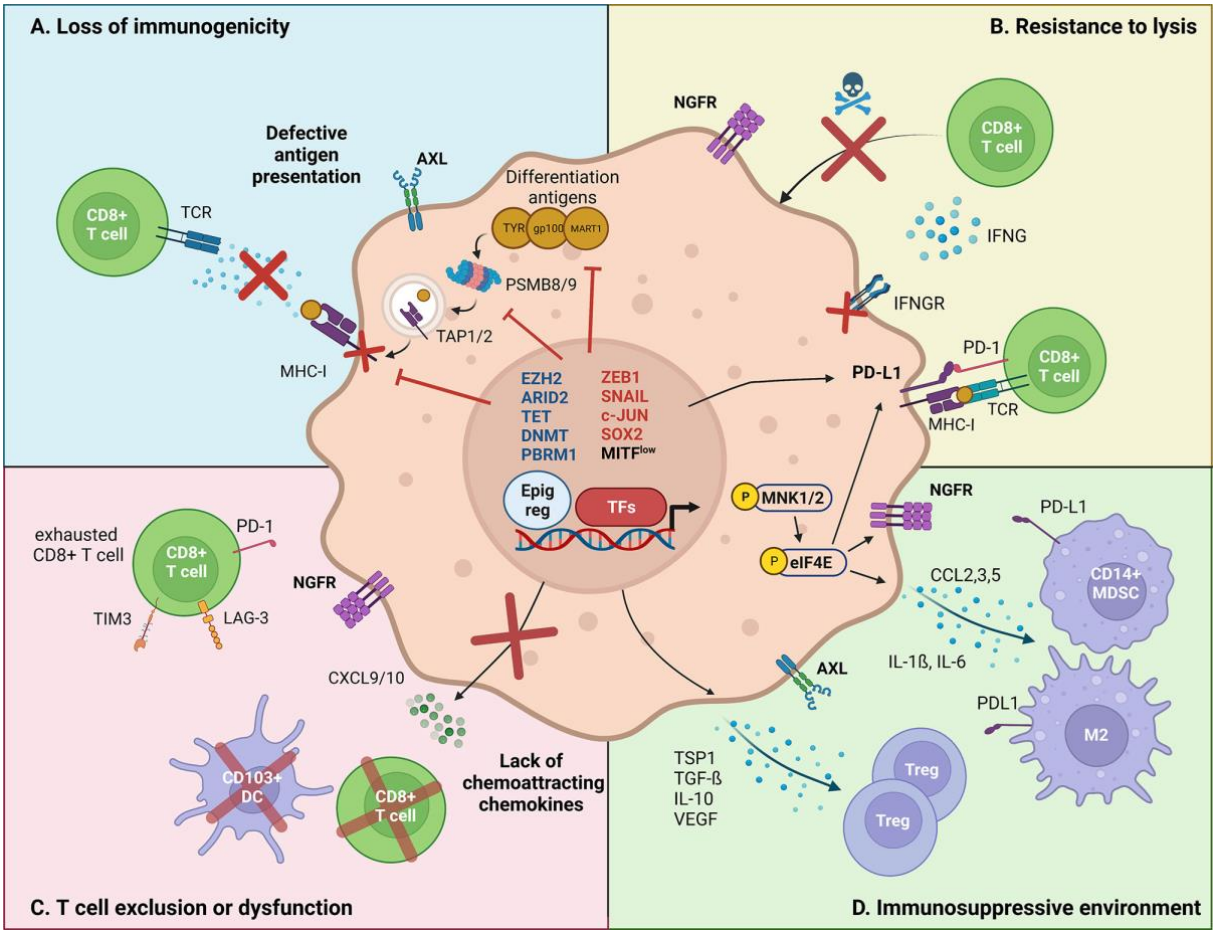


Figure 72 : overview of tumor escape mechanism from the immune system

7.9.2 Extravasation

In the bloodstream, cancer cells are subjected to strong hydrodynamic pressures that can kill them. They can surround themselves with platelets that allow them to survive the physical constraints of blood circulation. When cancer cells reach blood capillaries that perfuse an organ, they become blocked because their diameter is too large to pass. Indeed, they are larger than the diameter of the blood capillaries and the platelets that surround them hinder their passage even more. After getting stuck in the blood capillary of an organ, cancer cells can proliferate within that capillary and rupture it. They then leave the bloodstream and join the tissue that makes up the affected organ: this is called extravasation.

7.9.3 Colonization

The environment of the new tissue in which the cancer cells are located is not the same as that of the tumor from which they originate. This new environment is not conducive to tumor growth, and the small group of metastatic cancer cells forms a small, invisible tumor called a micrometastasis. To form a metastasis, these cells have to adapt to the new tissue, which takes them a long time: this process of metastasis formation is called colonization.

In the clinic, metastases can occur several months or years after a tumor has been removed from a patient, even though at the time of surgery, no other tumors were detectable. This shows that at the time of the excision of the tumor, the micrometastases were already in place, and that they took several months or years to adapt to their environment to form metastases. This phenomenon of adaptation of metastatic cancer cells to their new environment is the limiting factor for metastatic dissemination. Depending on where the cancer emerges, certain organs will be more favorable for the development of metastases because they provide a more advantageous environment for cancer cells from the start. For example, for breast cancer, the sites of metastasis are preferentially the bones and lungs, while for colon cancers, metastases to the liver are more frequent.

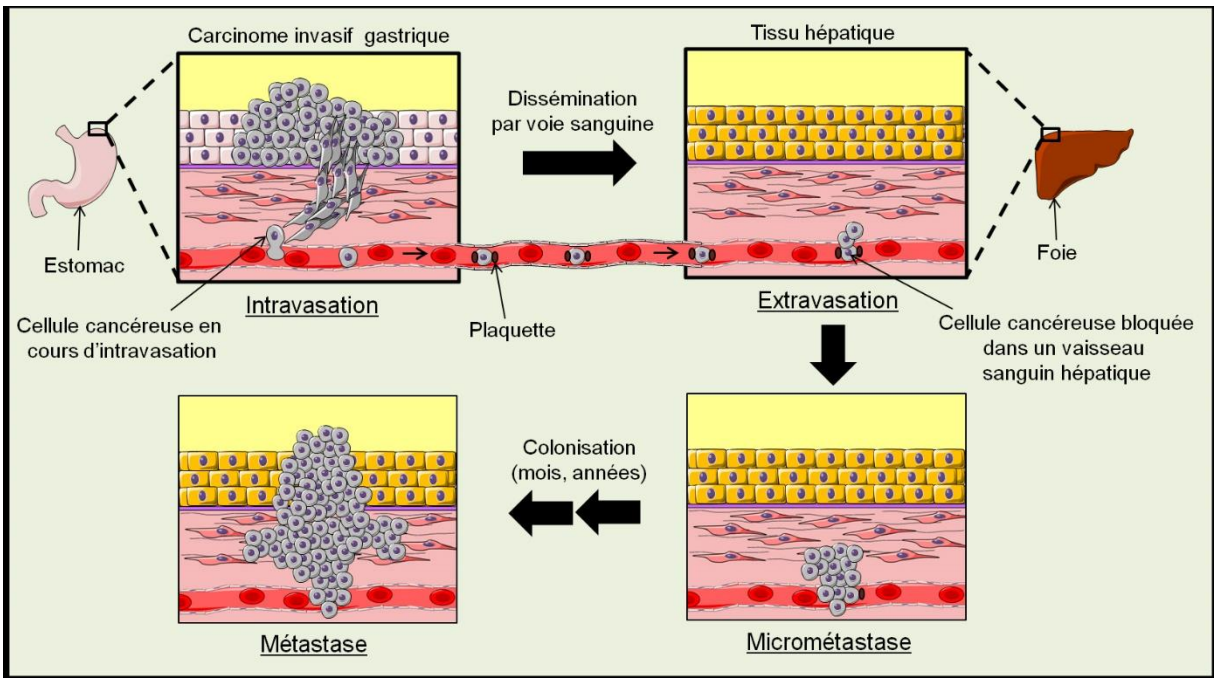


Figure 73: Mechanisms of Metastasis in Invasive Gastric Carcinoma: Intravasion, Extravasation, and Colonization.

The metastatic stage is the last stage of oncogenesis. Metastatic cancer is the most aggressive and life-threatening cancer.

8 GENES AND METASTASES

8.1 Introduction

The progression of tumor cells towards metastatic behavior is based on complex events that evolve within the cell progeny. For a given type of cancer, it is observed that a large primary tumor is more likely to generate metastatic variants. That said, even a small tumor can remain localized. This phenomenon of metastasis emergence can be understood by the mutation evolutionary model, which also explains cellular heterogeneity for various traits, including metastatic potential. A similar dynamic has been described by Goldie and Coldman, who deal with the emergence of chemotherapy resistance variants (Goldie, 1979).

Metastatic dissemination is the leading cause of cancer-related deaths. The mechanisms that give a tumor cell metastatic capacity are complex and still poorly understood. Some hypotheses suggest that cells with this potential exist from the earliest stages of the formation of the primary tumor. This potential may result from the deregulation of genes involved in dissemination. Metastasis suppressor genes (GSMs) play a key role, as they can inhibit the development of metastases without affecting the growth of the primary tumor. They often interact with signaling pathways, including G proteins and MAP kinases. Clinical studies show an inverse correlation between the expression of these genes in primary tumors and patient survival. GSMs modulate metastatic potential through epigenetic mechanisms, suggesting that a reactivation of their expression could be a promising therapeutic strategy.

In this synthesis, we will explore known GSMs, including NME (NM23), and the signaling pathways in which these genes may be involved.

Clinical studies linking the reduction of GSM expression to tumor aggressiveness parameters confirm these results. It is important to note that GSMs are not always present in metastatic signatures and are rarely subject to mutations or deletions. Their reduction in expression is often due to epigenetic mechanisms, such as promoter methylation. Currently, nearly 12 mobile phones have been identified, but this list is not exhaustive.

Table the different metastasis suppressor genes

Gène	Nom (Nom d'usage)	Date de découverte	Méthode d'identification	Localisation chromosomique	Type de tumeurs	Fonction
AKAP12 (SSeCKS)	*A-kinase PRKA anchor protein gravin 12 (Src-suppressed C kinase substrate)*	2001	HS	6q24-q25	Prostate	Inhibition de la formation des podosomes Action anti-angiogénique
BRMS1	*Breast cancer metastasis suppressor 1*	2000	TC + DD	11q13-q13.2	Mélanome, sein	Communication cellule/cellule (jonctions Gap) Partenaire du complexe répresseur transcriptionnel mSin3-HDAC
CD82 (KAI1)	*CD82 molecule*	1995	HS	11p11.2	Prostate, sein, mélanome	Atténuation de la voie de l'EGF-R Inhibition de l'activation de c-Met et de Src Interaction KAI1/DARC
CRSP3	*Cofactor required for Sp1 transcriptional activation subunit 3*	2003	TC + MA	6q22.33-q24.1	Mélanome	Régulateur de KiSS1
GDI2 (RhoGDI2)	*GDP dissociation inhibitor 2*	2002	MA	10p15	Vessie	Inhibition de l'activation de Rho-GTPases
KISS1	*KiSS-1 metastasis suppressor*	1996	TC + HS	1q32	Mélanome, sein	KISSpeptines : Ligands de GPR54 Répression transcriptionnelle de la MMP-9
MAP2K4 (MKK4)	*Mitogen-activated protein kinase kinase 4*	1999	TC + HS	17p11.2	Prostate, ovaire	Activation de p38 et de JNK
NDRG1 (Drg-1)	*N-Myc downstream regulated gene 1 (Differentiation-related gene 1)*	1997	DD	8q24	Prostate, côlon	Activation *via* MKK4
NME1 (NM23-H1)	*Non-metastatic cells 1 (Non-metastatic clone 23)*	1988	HS	17q21.3	Mélanome, sein, foie	Activité nucléoside diphosphate kinase Multifonctionnalité
PEBP1 (RKIP)	*Phosphatidylethanolamine binding protein 1 (Raf kinase inhibitory protein)*	2003	MA	12q24	Prostate, mélanome, côlon, foie	Inhibition de l'activation de ERK médiée par Raf
TXNIP (VDUP1)	*Thioredoxin interacting protein (Vitamin D upregulated protein)*	2003	TC + MA	1q21.1	Mélanome	Inhibiteur de la thiorédoxine Action anti-prolifération et pro-apoptotique

8.2 The NM23 gene

The NM23 gene (NM23-H1 in humans and NM23-M1 in mice) has been identified as a metastasis suppressor in a mouse model of melanoma, and it encodes an enzyme called nucleoside diphosphate kinase (NDPK), which synthesizes nucleoside triphosphates. Other genes in the NME family have been discovered, including NM23-H2 (NME2), which shares 88% identity with NM23-H1.

NM23-H1 expression is linked to carcinogenesis in two ways: on the one hand, early overexpression of NM23-H1 and NM23-H2 is observed in many human solid tumors compared to healthy tissue, suggesting that they promote the early stages of tumor progression. On the other hand, a loss of NM23-H1 is noted in the advanced stages of certain cancers, such as melanoma and breast, colon, and ovarian cancers, which is correlated with greater metastatic potential.

Studies show that NM23-H1 expression can inhibit the metastatic potential of aggressive tumor cells, and mice missing the NM23-M1 gene show increased lung metastases. Information on the role of NM23-H2 in metastasis is less clear and sometimes contradictory. The mechanisms of how NM23 influences metastatic potential remain to be elucidated, although it is suggested that NDPKs may play a role in the activation of GTP-binding proteins, which may explain their inhibitory effect on metastasis.

The figure illustrates the key role of NM23-H1 in various cell signaling pathways and the response to DNA damage. By being associated with receptor tyrosine kinases and integrins, NM23-H1 regulates pathways involved in cell proliferation across the Ras/Raf/MEK/ERK cascade, facilitating the inhibition of FAK (Focal Adhesion Kinase) autophosphorylation and participating in the degradation of KSR (Kinase Suppressor of Ras). In addition, NM23-H1 interacts with the CDC42 protein to modulate its activity, thereby inhibiting Rac1 and CDC42-mediated signals. In response to DNA damage, NM23-H1 associates with STRAP, influencing the activation of P53 and its interaction with MDM2, which is crucial for cell cycle regulation and apoptosis. Finally, NM23-H1 acts in a complex with proteins such as TAF-Iβ and HMG-2, contributing to DNA repair and induction of apoptosis via the granzyme A pathway. This scheme emphasizes the importance of NM23-H1 for the maintenance of cell integrity and the prevention of tumor progression.

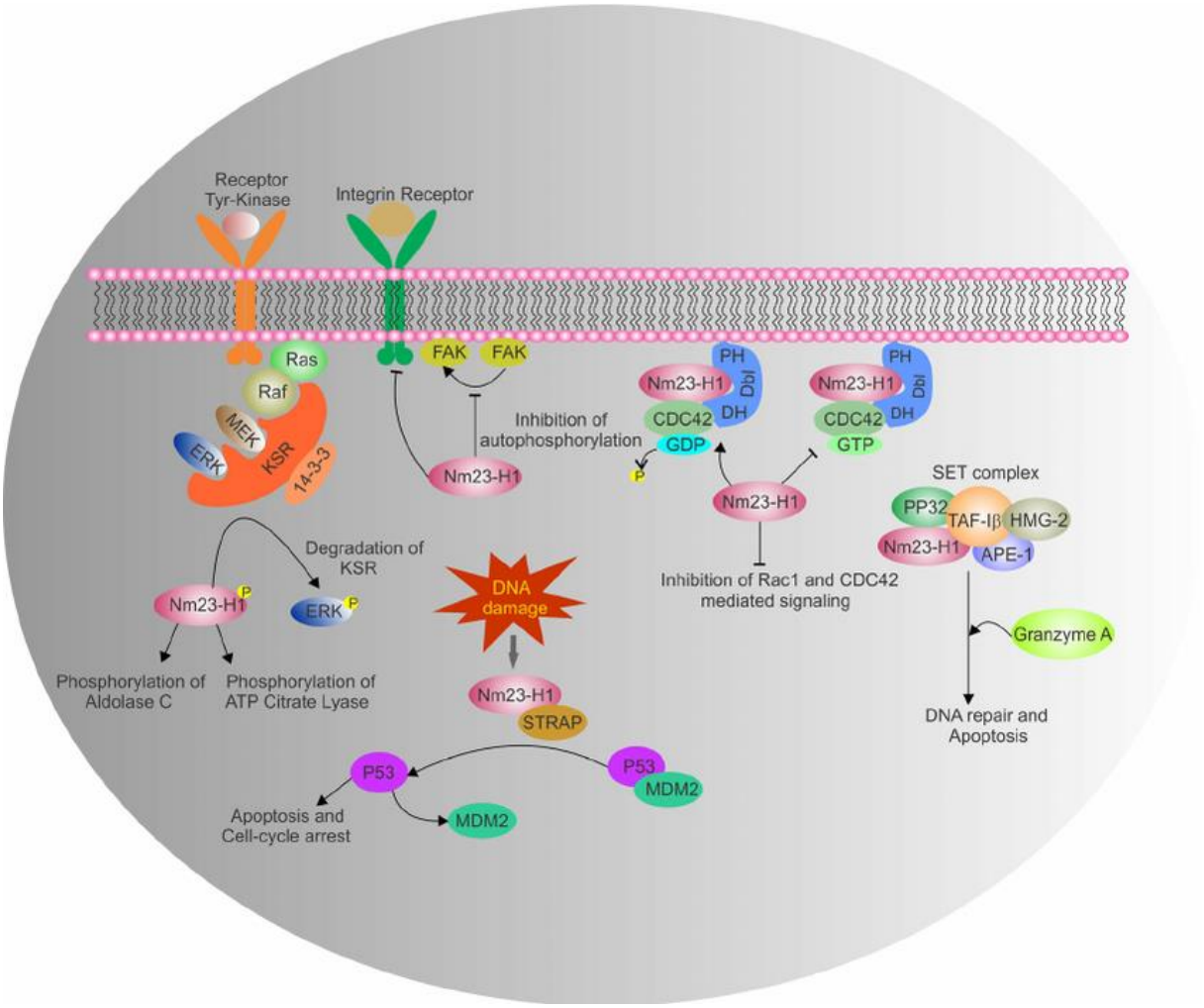


Figure 74: interactions and biological pathways involving the NM23-H1 gene, which plays a key role in the cellular response to DNA damage, as well as in the regulation of various signaling pathways.

8.3 Specificity of the organ

Several theories explain the ability of tumor cells to form metastases and invade certain tissues preferentially. Some suggest that this may be an intrinsic property of the primary tumor. Others explain the phenomenon by a filtering effect of the organ itself. There are also mechanistic explanations, according to which metastases would preferentially form in the first tissues where tumor cells would be trapped in the capillaries. In some cases, the production of chemo-attractor factors by target cells could be responsible for the tropism of tumor cells. In addition, it is possible that tumor cells attach themselves to many tissues but only grow in favorable environments, a complex explanation but supported by experimental evidence and defended by Paget's theory.

Another theory holds that tumor cells send signals through the body and that only certain tissues respond by preparing for the arrival of tumor cells.

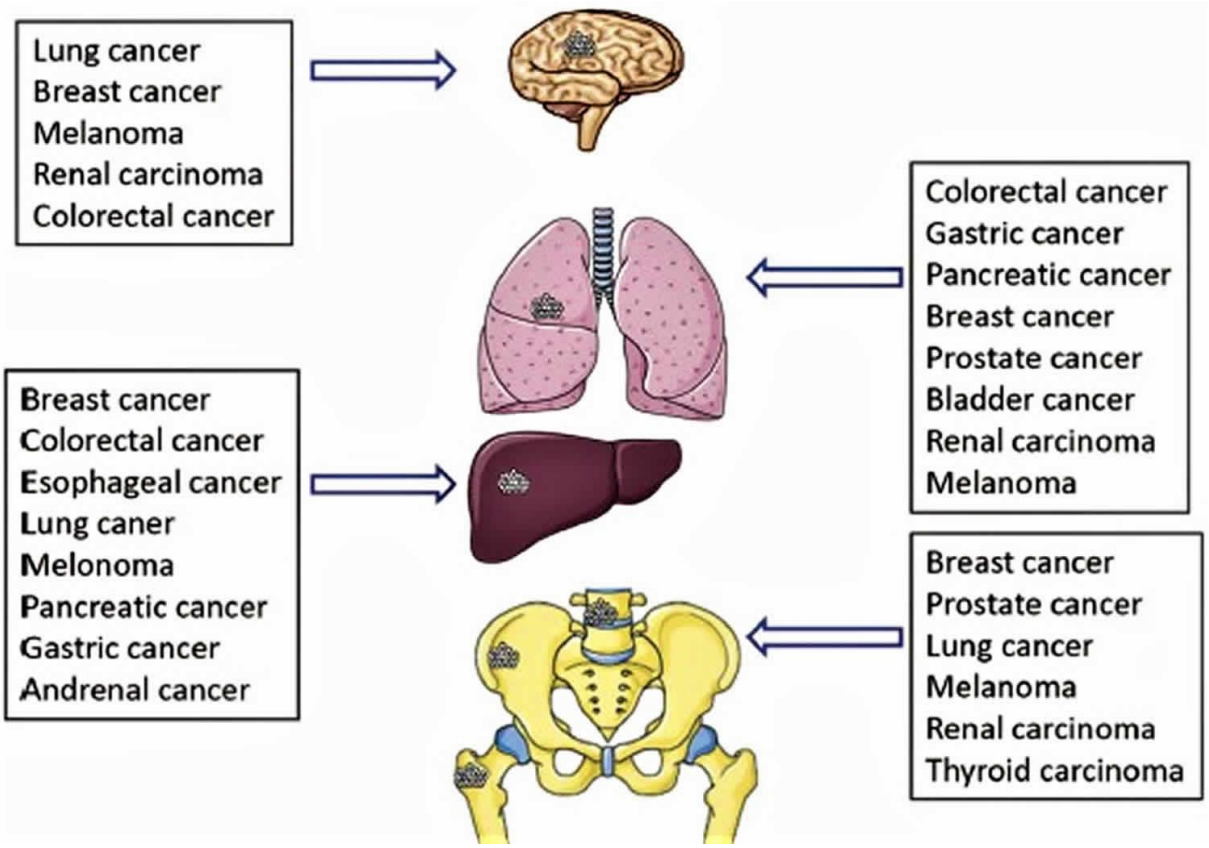


Figure 75 : organ specifity in cancer metastasis

8.4 Cell Signaling Pathways and Cancer

Cellular communication is essential to the life of multicellular organisms. The information transmitted from one cell to another corresponds to six main types of instructions to be carried out, antithetical two by two: reproduce or differentiate; remain attached or migrate; survive or die. The cancer cell is a genetically unstable cell, capable of exploring the functions of the entire genome and taking advantage of any proliferative or migratory advantage to select it and transmit it to its offspring. All the signaling pathways involved in proliferation and differentiation, adhesion and migration, survival and death, can serve as support for oncogenic alterations. Thanks to many genetic or epigenetic mechanisms, the cancer cell is able to take advantage of this signaling, divert it from its purposes, and use it to proliferate, to migrate, to survive. In this sense, it has been said that cancer is a disease of cell signaling.

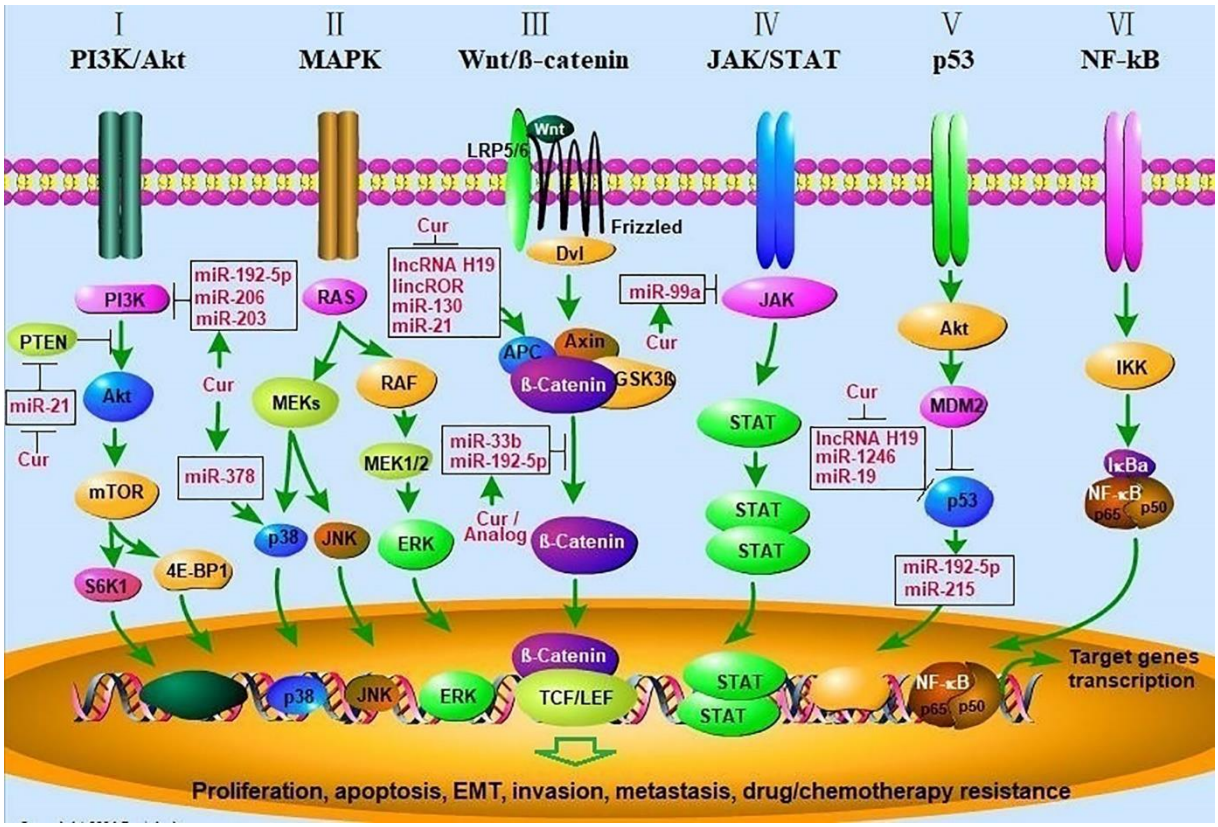


Figure 76: out the different pathways involved in cancer

These alterations can precisely be the subject of therapeutic targeting: it is from this observation that the concept of targeted therapy was born. We present here some examples of this therapeutic targeting at the level of a major proliferation pathway, showing how we have been able to identify and characterize relevant targets, invent original therapeutic tools, and discover the resistance mechanisms that are put in place and hinder the success of targeted therapies.

8.5 The pathway of mitogen-activated protein (MAP) kinases: RAS, RAF, MEK, and ERK

The Ras signaling pathway is involved in the regulation of cell proliferation. Mutations in RAS genes, especially KRAS, are among the most common in cancer and result in constitutive activation of the RAS protein, keeping it constantly "turned on", independent of external signals. This causes continuous stimulation of the proliferation and survival pathways, promoting tumor development. KRAS mutations are common in cancers such as pancreatic cancer (present in nearly 90% of cases), colorectal cancer, and non–small cell lung cancer, while NRAS and HRAS mutations are found in other types of cancers, such as melanomas. Cancers associated with these mutations often prove difficult to treat, showing resistance to

targeted therapies and thus becoming more aggressive and less receptive to conventional treatments.

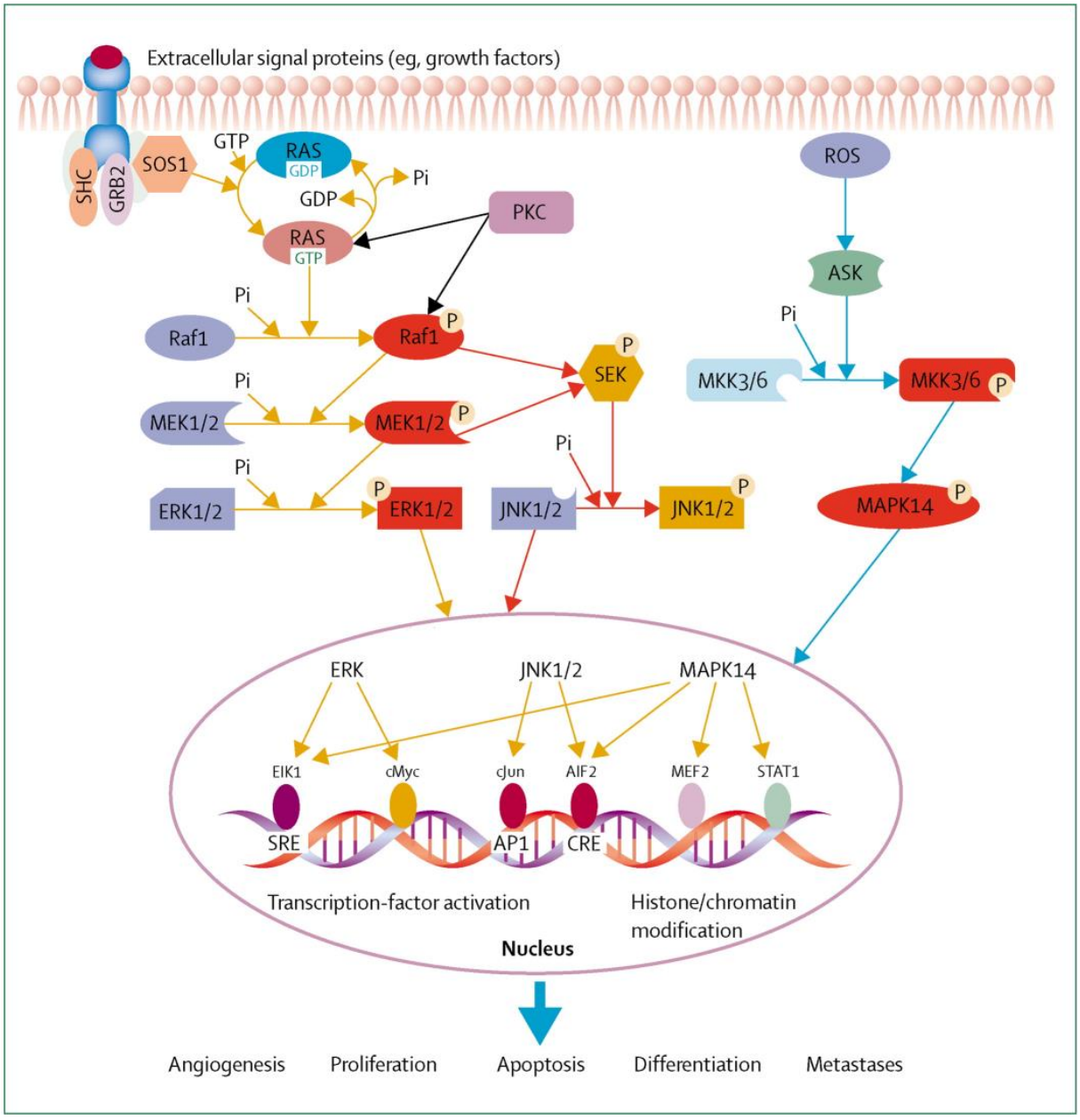


Figure 77: the mapk way

8.6 The pathway PI3K/Act/mTOR

This pathway is often activated in various types of cancers. It regulates processes such as cell survival, growth, and protein synthesis. Mutations in the PIK3CA genes (which encode a subunit of phosphoinositide 3-kinase) or activation of growth factor receptors, such as the

epidermal growth factor receptor (EGFR), can lead to excessive activation of this pathway.

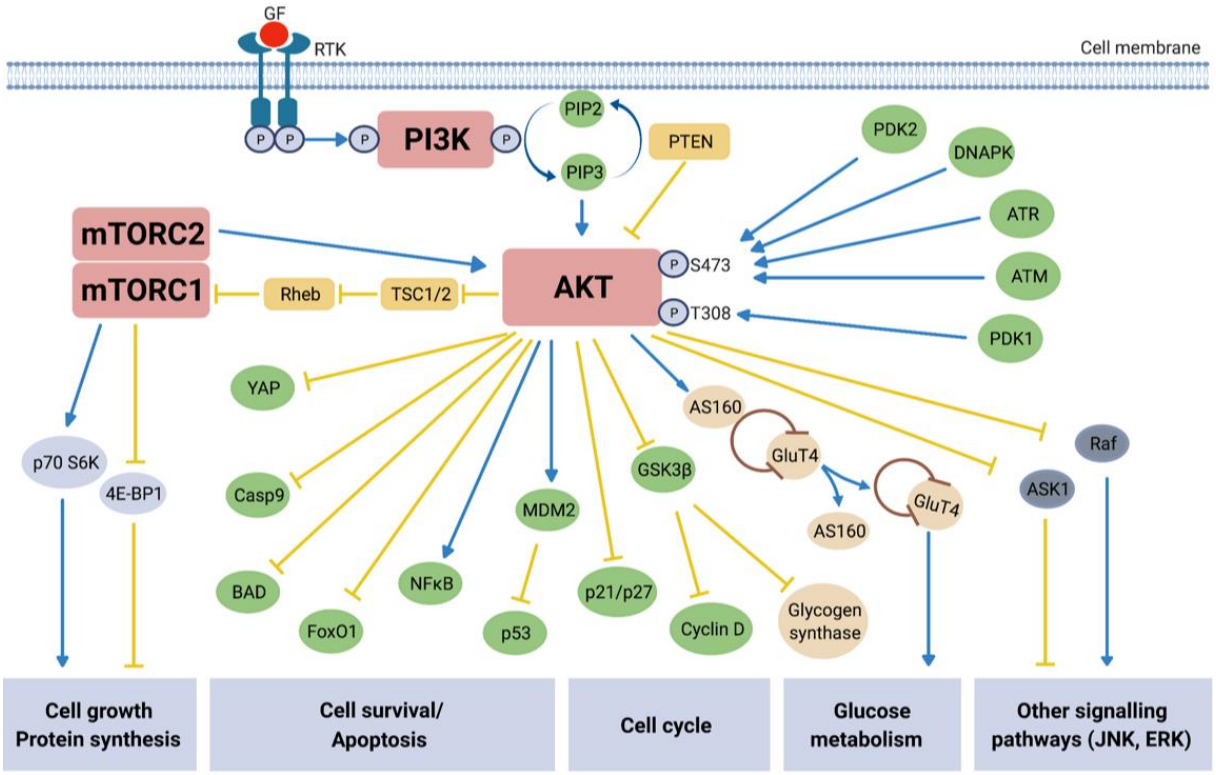


Figure 78 : Overview of PI3K-AKT-mTOR signaling pathway

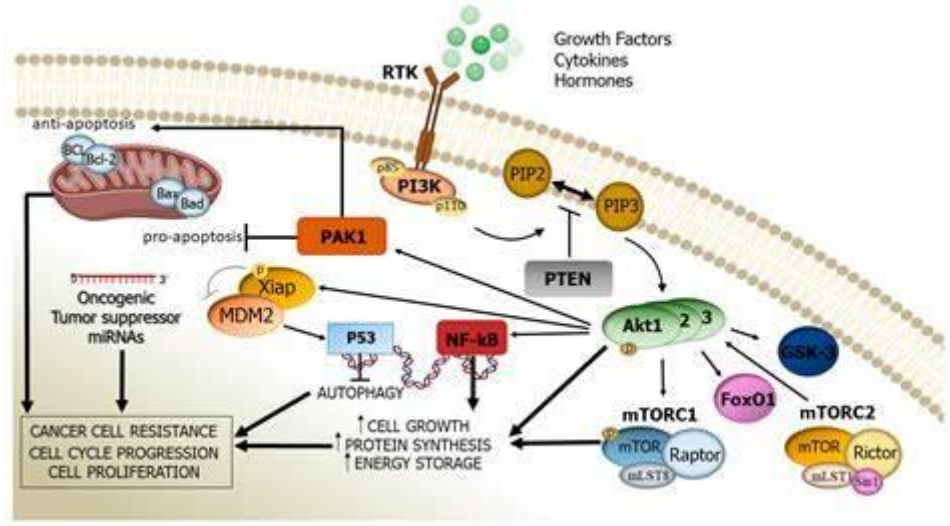


Figure 79: involvement of PI3K/Akt/Mtor signaling pathways in the development of cancers.

8.7 HFR-β pathway

Although this pathway may act as a tumor suppressor in its early stages, mutations and alterations in this pathway may also promote tumor progression in advanced stages. It is involved in the regulation of cell growth, migration, and apoptosis.

8.8 Wnt/β-catenin pathway

Involved in the regulation of embryonic development and tissue homeostasis, the Wnt/β-catenin pathway is an essential signaling pathway that regulates cell proliferation, differentiation, and survival. Mutations in key genes such as APC (adenomatous Polyposis Coli) and β-catenin can lead to aberrant activation of this pathway, leading to uncontrolled cell proliferation and tumor stem cell survival. This dysregulation is particularly associated with colorectal cancers, but it also plays a role in other types of cancers, such as breast cancer and melanoma. Due to its involvement in tumorigenesis, the Wnt/β-catenin pathway is considered a promising target for cancer therapies, although the development of such treatments poses challenges in terms of specificity and side effects. (figure).

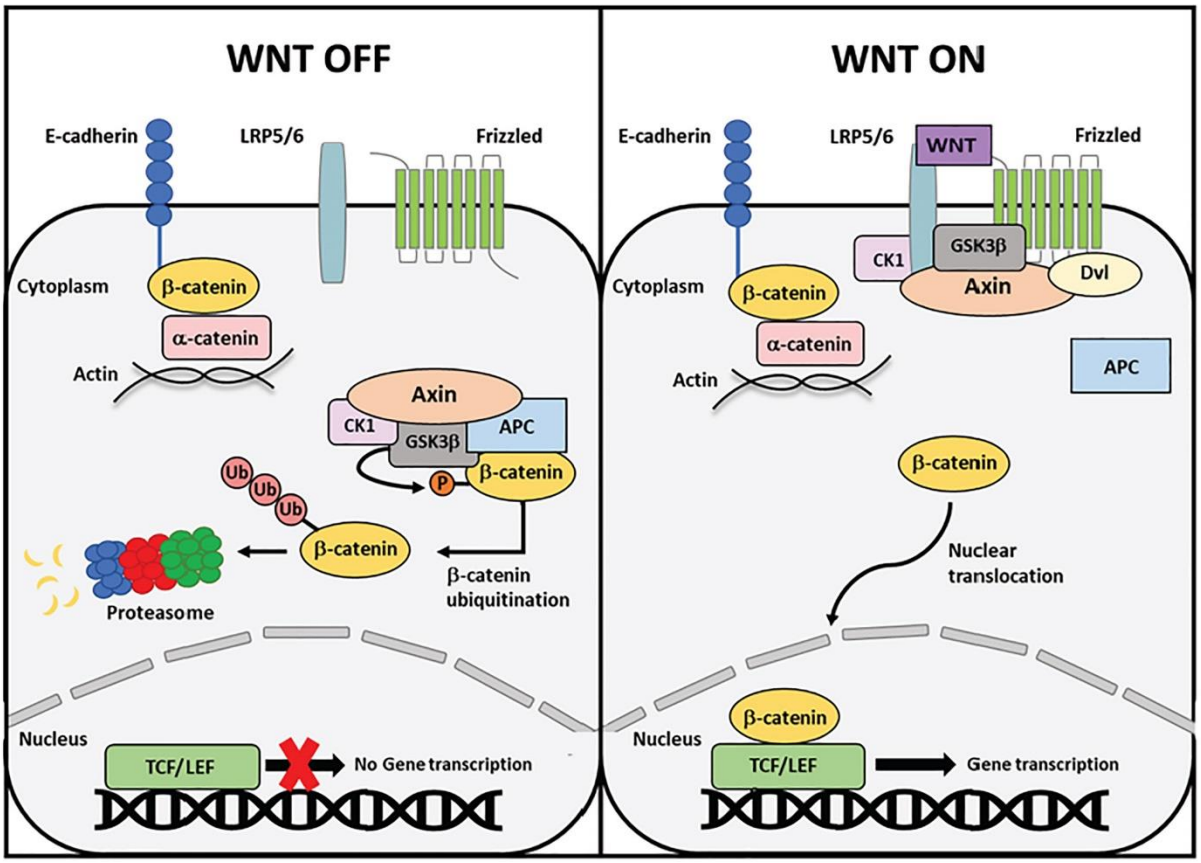


Figure 80: the wnt catenin signaling pathway

8.9 Notch Way

Notch signaling plays a crucial role in the regulation of cell differentiation and survival. Mutations in this pathway can contribute to some leukemias and lymphomas. Aberrant activation of the Notch can lead to aggressive tumor behaviors.

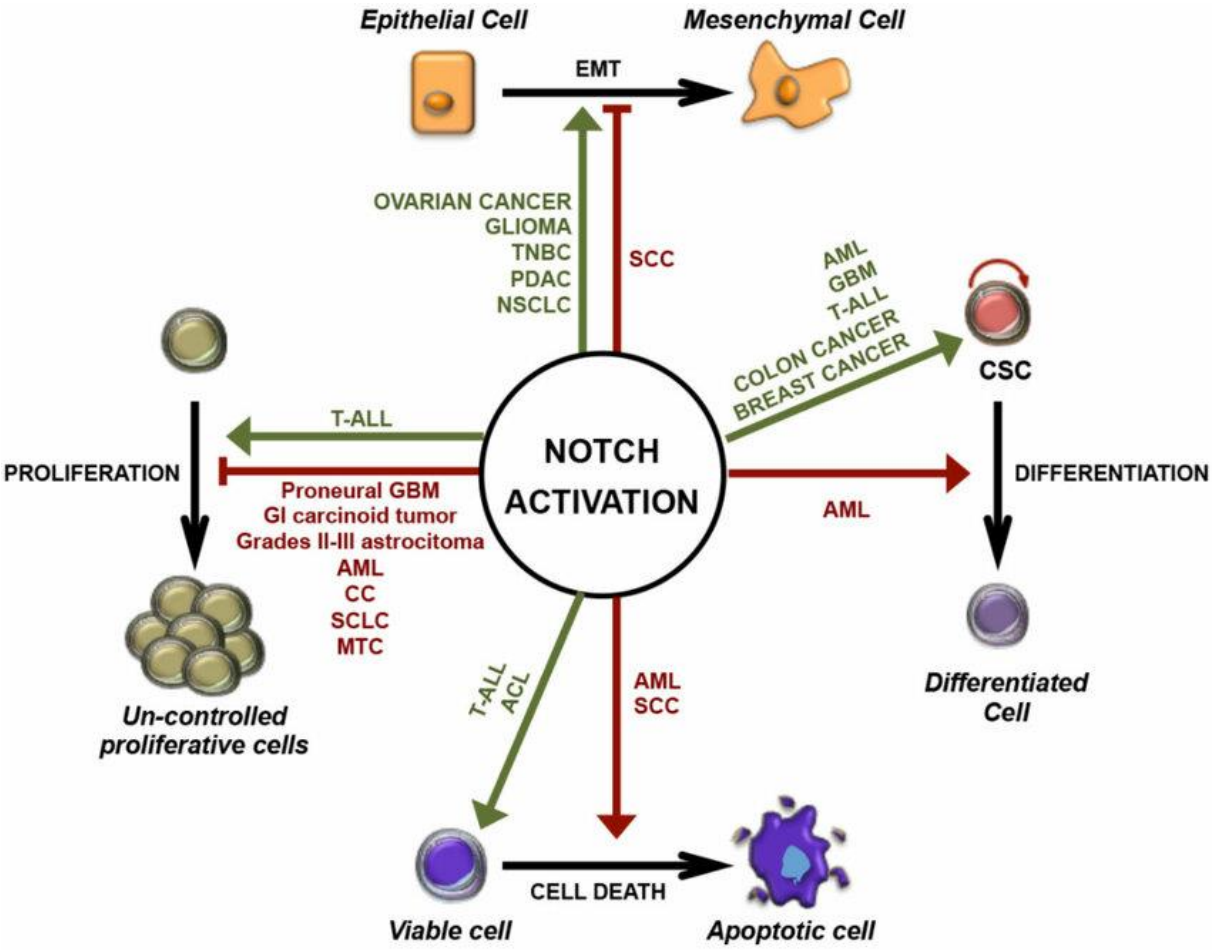


Figure 81: Pleiotropic functions of Notch activation in cancer. Schematic representation of oncogenic (green) and tumorsuppressive (red) roles of Notch signaling in different cancers: stimulation or inhibition of uncontrolled proliferation; regulation of epithelial-mesenchymal transition (EMT); induction of differentiation or maintenance of cancer stem cells (CSCs); promotion of cell survival or cell death. ACL: lung adenocarcinoma; AML: acute myeloid leukemia; CC: cervical cancer; GI: gastrointestinal; GBM: glioblastoma multiforme; MTC: medullary thyroid carcinoma; NSCLC: non-small-cell lung cancer; PDAC: pancreatic ductal adenocarcinoma; SCLC: small-cell lung cancer; SCC: squamous cell carcinoma; T-ALL: T-cell acute lymphoblastic leukemia; TNBC: triple-negative breast cancer.

8.10 The Pathway JAK/STAT

This pathway is important for the immune response and cytokine regulation. Mutations and chronic activation of this pathway are often seen in hematological cancers, such as myelodysplasia and leukemia.

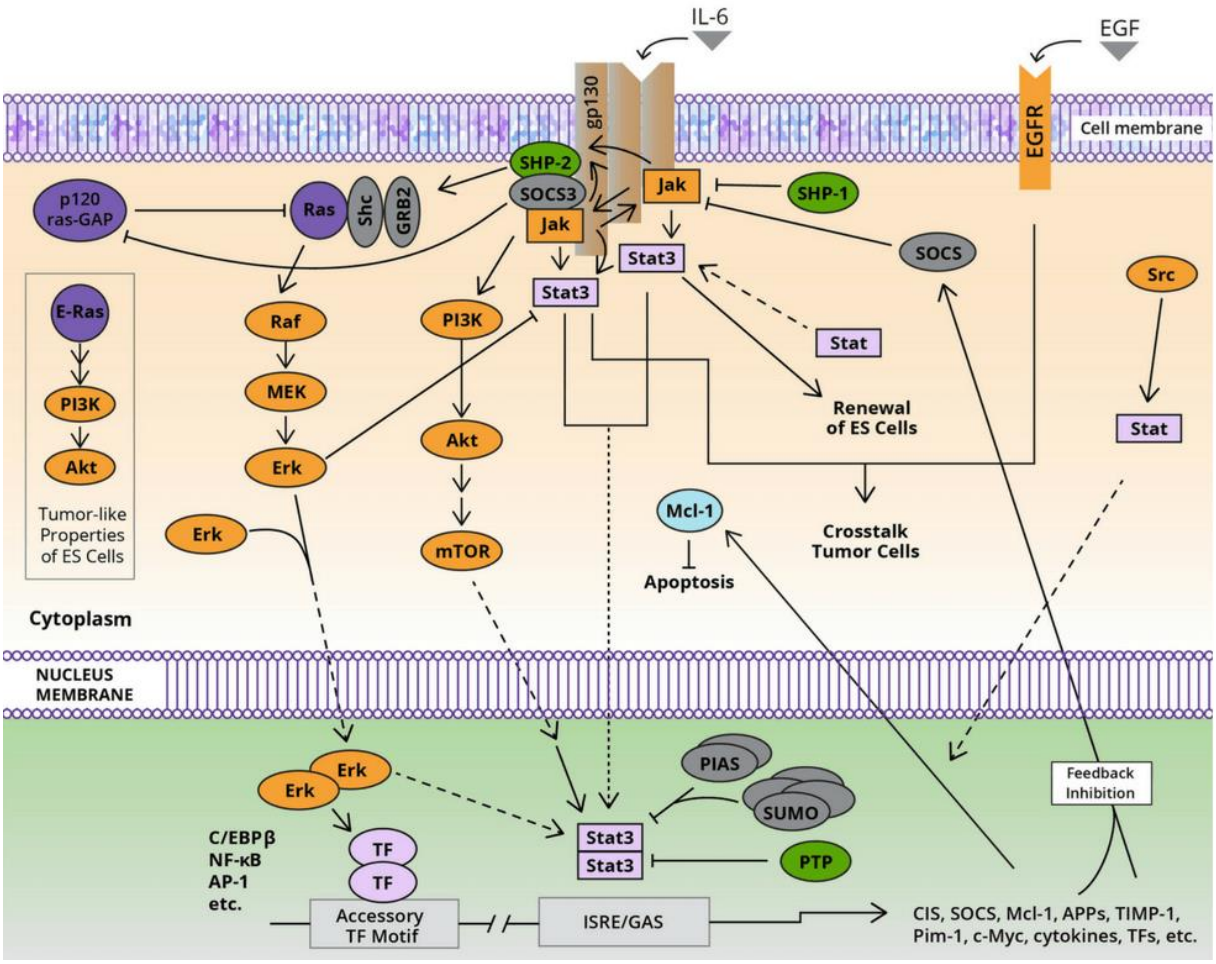


Figure 82 : Jak/Stat pathway cancer

8.11 Her2

HER2 (Human Epidermal Growth Factor Recptor2) is a protein that plays an important role in cell growth and division. It is a receptor that belongs to the EGFR family of epidermal growth factor receptors

When HER2 forms a dimer with another receptor, the activated receptor triggers an intracytoplasmic signaling cascade. This activation stimulates several signaling pathways, including The PI3K/Akt pathway, which promotes cell survival and growth and the MAPK/ERK pathway, involved in cell proliferation and differentiation.

HER2 amplification or overexpression is associated with aggressive cancers, including breast cancer and, to a lesser extent, gastric cancer. In breast cancer, for example, about 20 to 30% of tumors express high levels of HER2, which is a factor in poor prognosis and resistance to standard treatments.

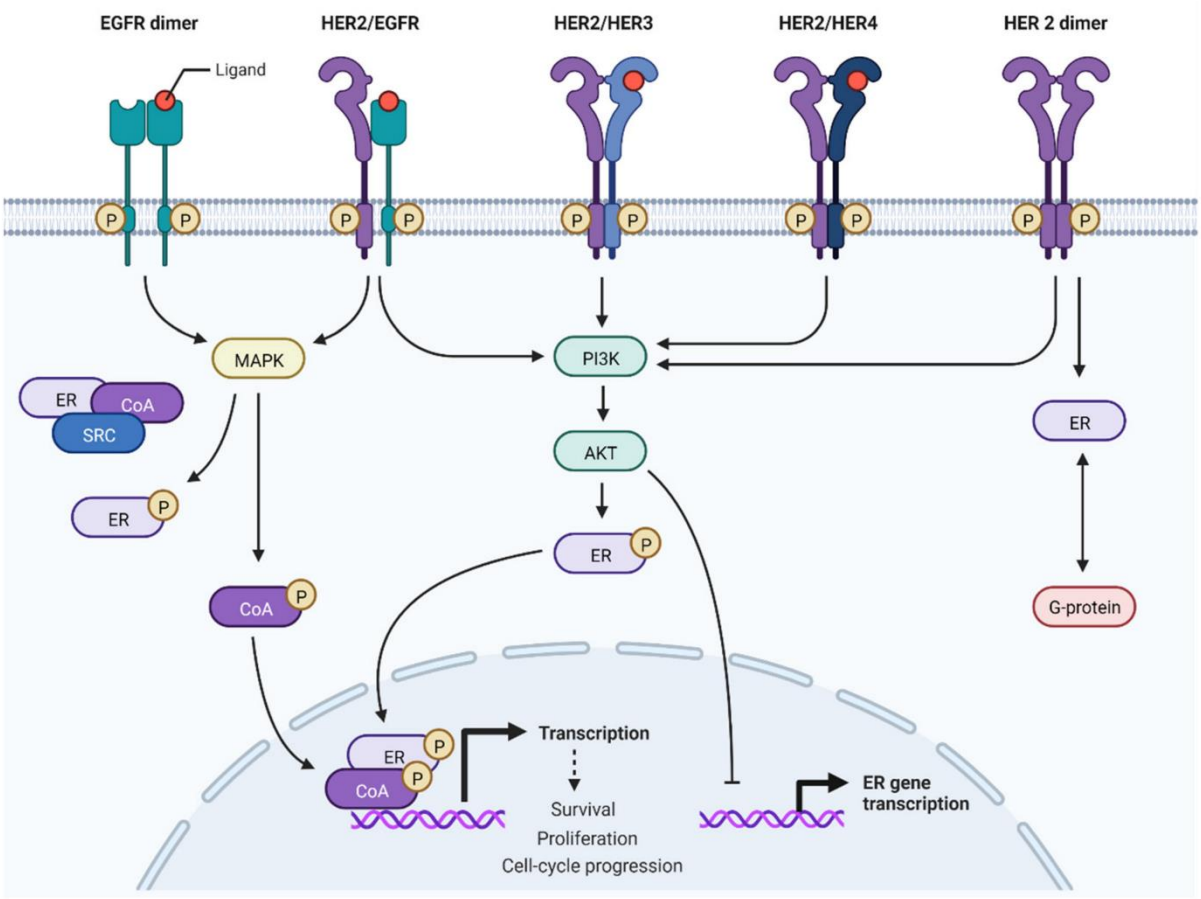


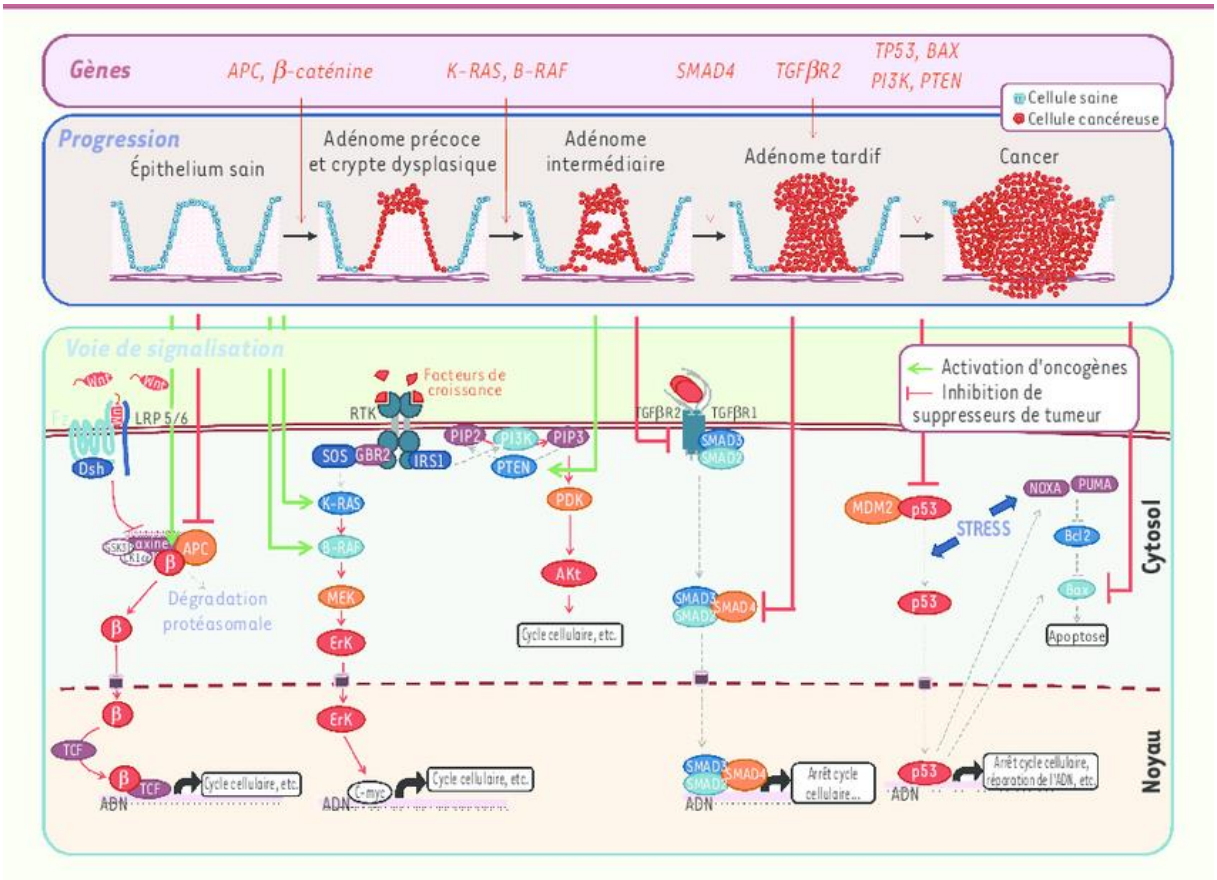
Figure 83 : Signaling Pathways Involving HER2 and EGFR Dimerization. visual representation underscores the critical roles of these receptors in cancer biology and therapeutic targets..

9 EXAMPLES OF THE MOLECULAR MECHANISM OF CERTAIN CANCERS

9.1 Cancer colorectal

Colorectal cancer is one of the most common cancers, accounting for about 10% of all new cancer cases diagnosed worldwide. It mainly affects people over the age of 50, although its incidence is increasing among young adults in some regions. In 2020, colorectal cancer was responsible for approximately 1.9 million new cases and 935,000 deaths, making it the second leading cause of cancer mortality. Risk factors include a high-fat, low-fiber diet, obesity, smoking, excessive alcohol consumption, and a family history of colorectal cancer. Early detection, through methods such as colonoscopy, is essential to reduce mortality by allowing for early detection and treatment.

The figure illustrates the developmental pathways of colorectal cancer, detailing two main mechanisms: chromosomal instability (CIN) and microsatellite instability (MSI). In the CIN pathway, mutations such as APC inactivation and KRAS mutation result in progressive changes from the normal mucosa to carcinoma. The MSI pathway, on the other hand, is characterized by mutations in repair genes (MMRs) and MLH1 methylation, which increases errors during DNA replication. These processes highlight the molecular mechanisms leading to the formation of malignant tumors.



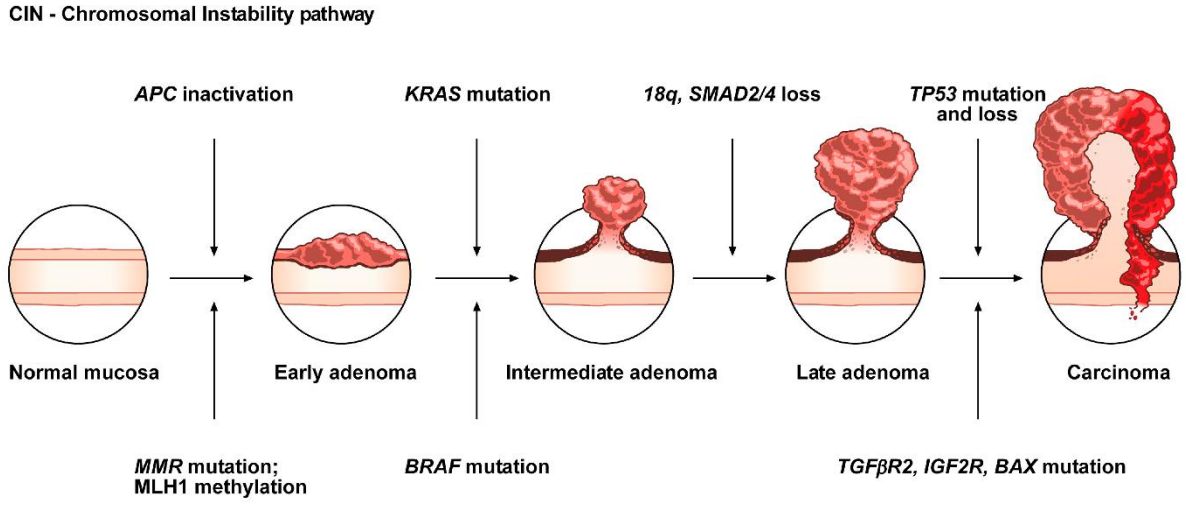


Figure 84: Molecular model for the evolution of colorectal cancer

9.2 Lung cancer

Lung adenocarcinoma is the most commonly diagnosed type of lung cancer, accounting for about 40% of all cases. Among the driver mutations identified in this cancer, the most common is KRAS, present in 25% of cases, followed by EGFR-sensitizing mutations at 15% (figure). Other notable mutations include ALK (Anaplastic Lymphoma Kinase) (activator of the MAPK and PI3K-AKT signaling pathways) and BRAF V600E (proto-oncogenic B-Raf), at 7% and 10%, respectively. About 31% of cases do not have clearly identified driver mutations, highlighting the genetic diversity of cancer and the challenges associated with its treatment. The incidence of lung adenocarcinoma continues to increase, in part due to smoking and exposure to environmental carcinogens, including air pollution. Screening and the search for targeted mutations are essential to improve patient care.

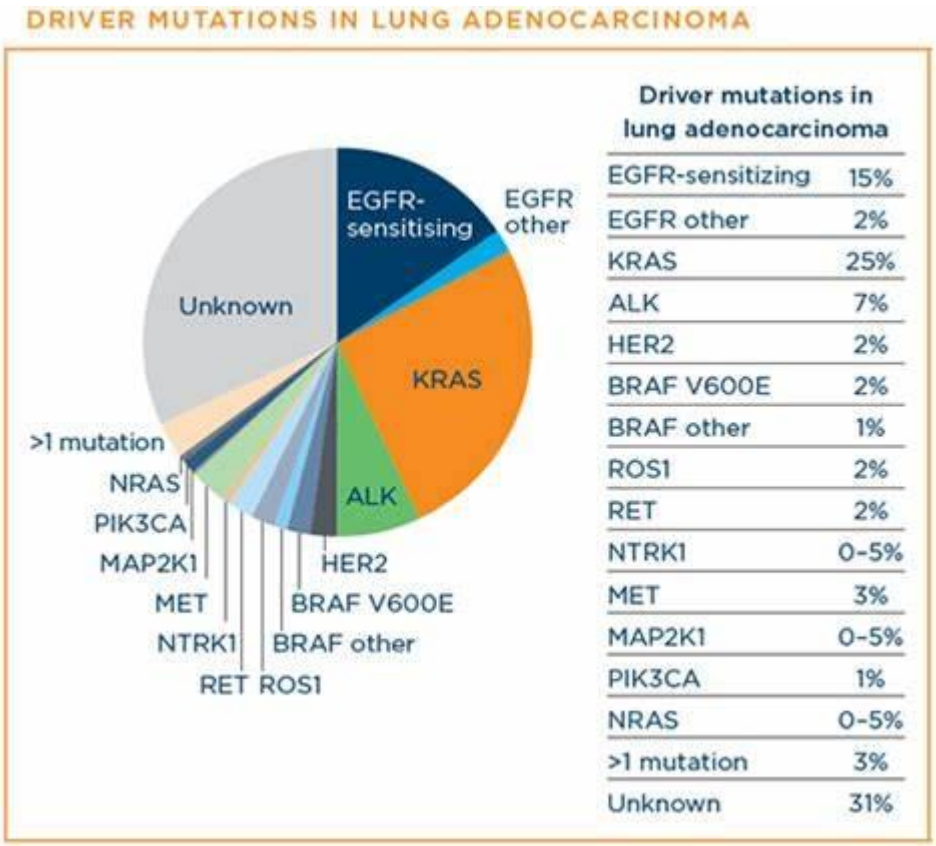


Figure 85: Driver Mutations in Lung Adenocarcinoma: Distribution and Frequency

9.3 Breast Cancer

Breast cancer is one of the leading causes of death in women worldwide. According to the World Health Organization, it accounts for about 25% of all cancer cases diagnosed in women. Its prevalence is particularly high in developed countries, but rates are also increasing in developing regions. Risk factors include advanced age, a family history of breast cancer, specific genetic mutations, as well as environmental and hormonal factors.

The pie chart shows the distribution of mutations in genes linked to breast cancer, with a focus on the BRCA1 and BRCA2 genes, which are among the main genetic determinants in breast cancer predisposition. BRCA1 reveals the highest number of mutations (40), highlighting its central role in the development of the disease. BRCA2 follows, with 23 mutations, also indicating its significant importance. Other genes such as TP53, RAD51D, PALB2, NBN, MSH6, MRE11, CHEK2, BRP1, and BARD1 (involved in DNA damage detection, recombination repair, and ring regulation) show less frequent mutations (between 1 and 5), suggesting a contribution to predisposition while being less predominant than BRCA1 and BRCA2.

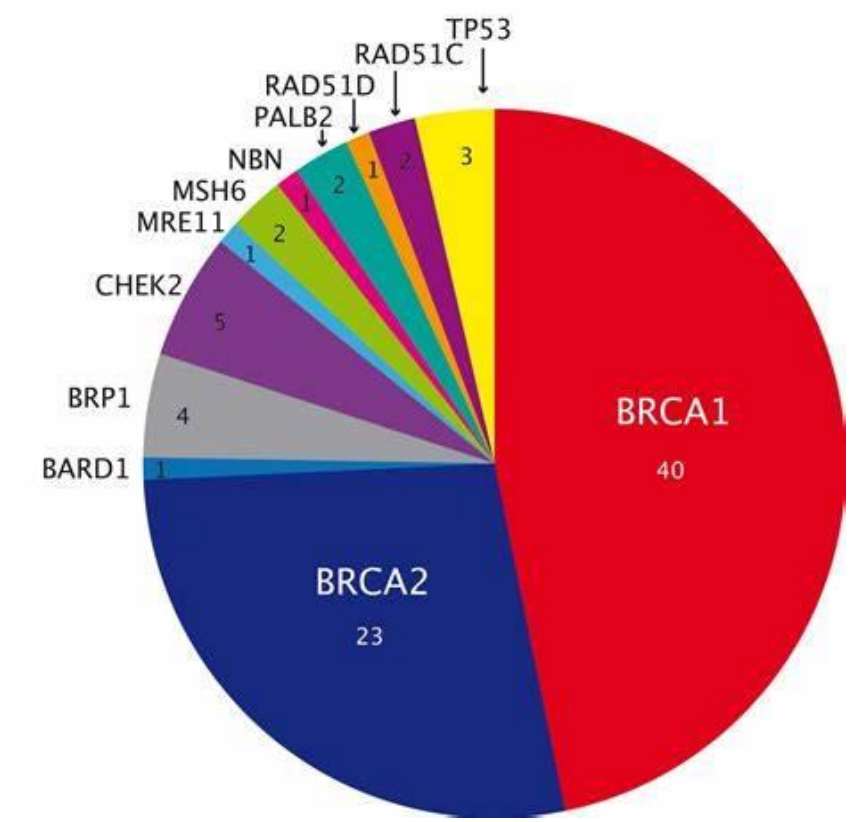


Figure 86: the distribution of mutations in genes linked to breast cancer

Indeed, BRCA1 and BRCA2 are essential genes for DNA repair and the maintenance of genetic stability, playing a crucial role in the prevention of cancer, especially breast and ovarian cancer. The proteins they encode are involved in homologous recombination repair, correcting DNA damage. The figure illustrates their functions, highlighting the structure of these proteins: BRCA1 has a RING domain and BRCT domains that allow it to interact with other proteins to detect DNA damage. BRCA2, on the other hand, has BRC repeats, an alpha domain, and OB domains, which facilitate its interaction with repair proteins (Figure A). BRCA1 initiates the DNA damage response by interacting with 53BP1 and CTIP, to initiate homologous recombination repair. BRCA2 then intervenes in this process by recruiting RAD51, a key player in this repair. In addition, these proteins regulate the cell cycle by ensuring that cells do not progress towards division before damage is repaired, with BRCA1 participating in mitotic spindle assembly and BRCA2 involved in critical stages of mitosis. Thus, BRCA1 and BRCA2 are fundamental to maintaining genetic integrity and preventing cancer development by ensuring precise damage repair and chromosomal stability.

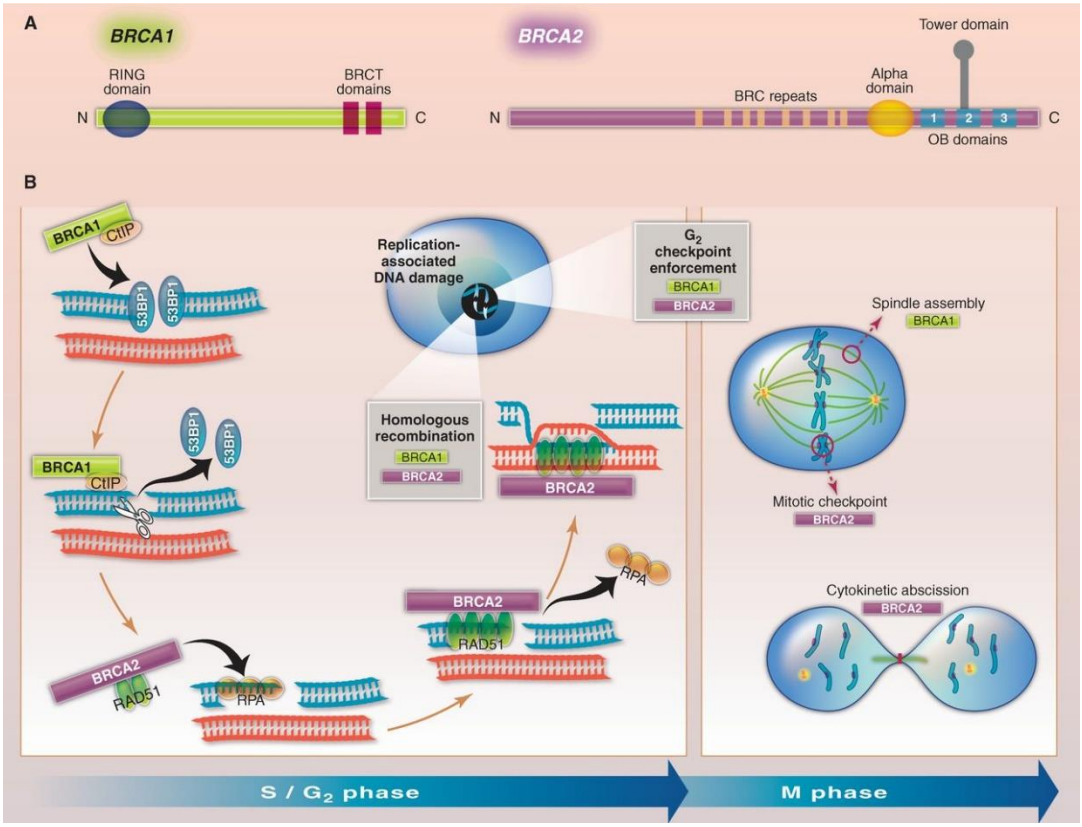


Figure 87: Functions and mechanisms of action of BRCA1 and BRCA2 proteins in DNA repair and cell cycle regulation

Molecular subtypes of breast cancer include Luminal A (40%) with hormone receptor-positive and a good prognosis as they can usually respond to targeted hormone treatments that block the

effects of estrogen or progesterone or reduce their production. ; Normal-like (2-8%) with a variable prognosis; Luminal B (20%) which can be positive or negative for hormone receptors, with an intermediate prognosis; HER2-enriched (10-15%) which is negative for hormone receptors and positive for HER2, having a generally poor prognosis; and Basal-like/Triple Negative (15-20%), which is negative for all three receptors, with the worst-case prognosis (Figure).

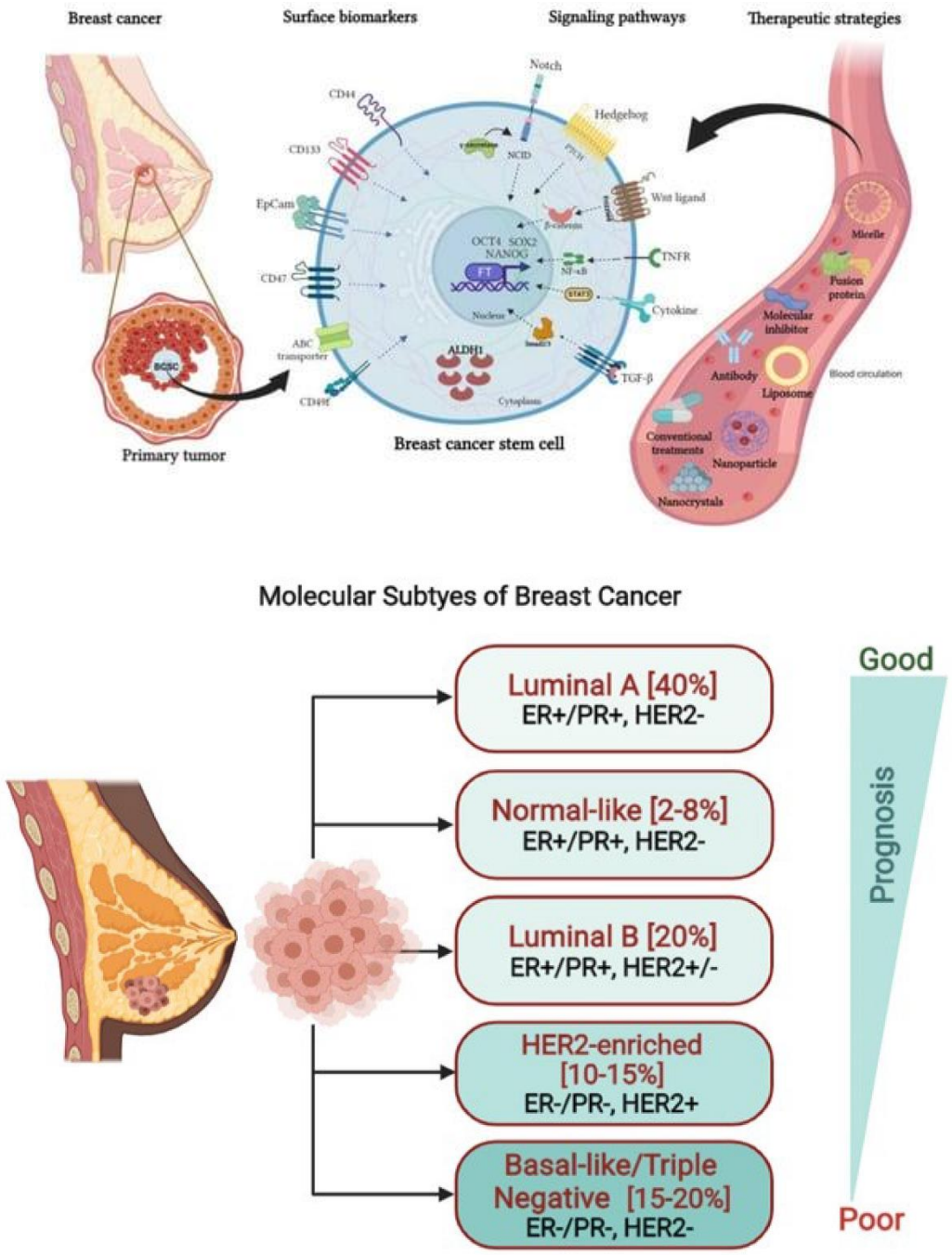


Figure 88: Lists the molecular subtypes of breast cancer, listed with their associated characteristics and prognosis.

These classifications help determine the appropriate treatment and assess the prognosis of patients (figure)

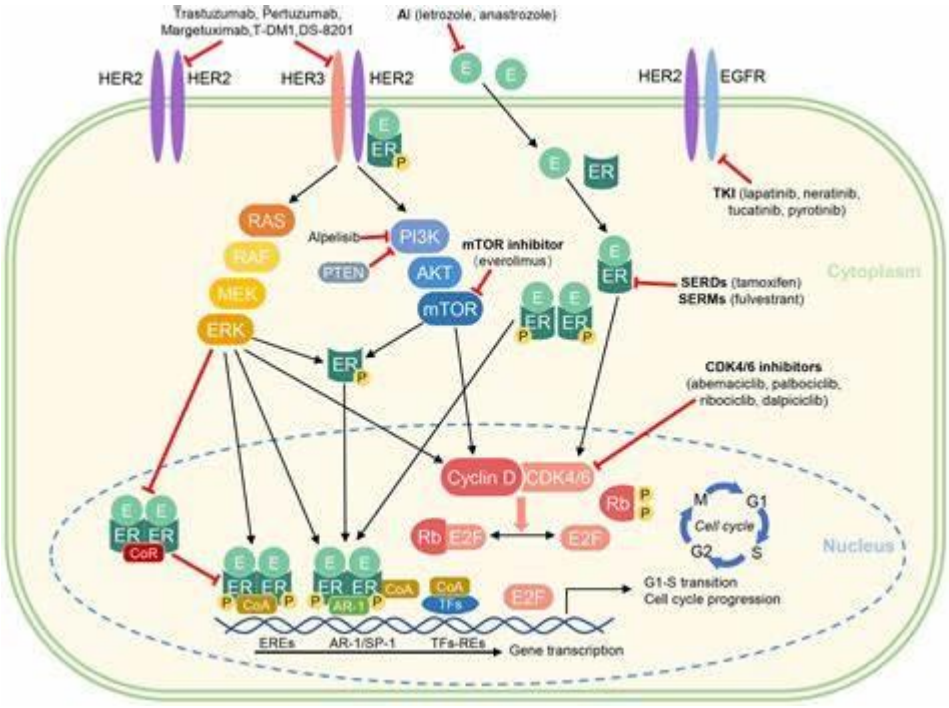


Figure 89: Description of signaling pathways in breast cancer and therapeutic targets

All of these elements illustrate the complexity of the genetic factors associated with breast cancer, highlighting the importance of thorough genetic screening and evaluation for those at risk, in order to optimize the prevention and management of the disease.

9.4 Uterine Cancer

Uterine cancer, especially cervical cancer, is often associated with human papillomavirus (HPV) infection, making it one of the cancers where initiation is not primarily due to spontaneous genetic mutations. HPV, especially high-risk types like HPV-16 and HPV-18, is responsible for the vast majority of cervical cancer cases.

Cervical cancer is one of the leading causes of cancer death in women worldwide. According to the World Health Organization (WHO), there are about 570,000 new cases each year. Developing countries are particularly affected, due to limited access to testing and vaccination programs.

At the molecular level, HPV infection results in the expression of oncogenic viral proteins, including E6 and E7. These proteins interfere with cell cycle control mechanisms:

E6 protein: It interacts with the p53 protein, an important tumor suppressor, promoting tumor degradation. By degrading p53, E6 inhibits apoptosis and eliminates DNA damage response mechanisms, allowing infected cells to survive even in the presence of mutations and abnormalities. E6 also induces the degradation of certain proteins with the PDZ domain (Post-synaptic density protein, Drosophila disc large, and ZO-1), influencing various cellular processes (cell signalization, cell localization, interaction between various proteins, etc.). etc). It also stimulates the expression of hTERT (human telomerase), promoting cellular immortality by maintaining telomere length.

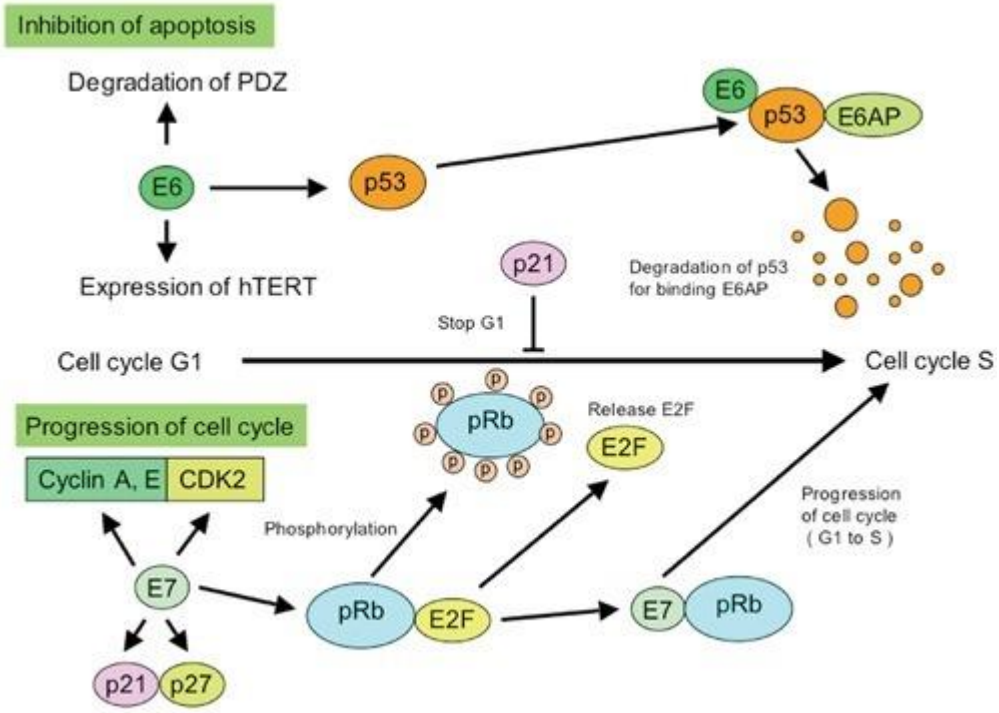


Figure 90 : Schematic presentation of the HPV viral oncoprotein E6 and E7

E7 protein: It binds to and stimulates the breakdown of the RB (Retinoblastoma) protein, which is a key regulator of the cell cycle. This releases E2F transcription factors, allowing inappropriate cell cycle progression from the G1 to S phase, promoting cell proliferation.

These interactions lead to uncontrolled cell proliferation and progressive genomic changes, facilitating the evolution to a cancerous phenotype. In summary, although cervical cancer is associated with genetic alterations, its initiation is mainly induced by HPV infection, illustrating the importance of viral factors in carcinogenesis.

9.5 Prostate cancer

Prostate cancer is one of the most common forms of cancer in men, developing from the cells of the prostate, a gland in the male reproductive system. Its prevalence is particularly high in developed countries, with about 1 in 8 men being diagnosed in their lifetime. The main risk factors include age, family history, ethnicity, and certain hormonal and dietary aspects. Although prostate cancer is often detected at an early stage and can be treated effectively, it remains the second leading cause of cancer death in men, after lung cancer. This underscores the importance of early detection and ongoing research into the factors that influence the disease. Several gene mutations are involved in this cancer. The figure shows the distribution of cancer-associated gene mutations, highlighting the relative importance of different genes. All of these genes are involved in DNA repair and cell cycle regulation, processes that are essential for maintaining genetic integrity and preventing the development of cancers.

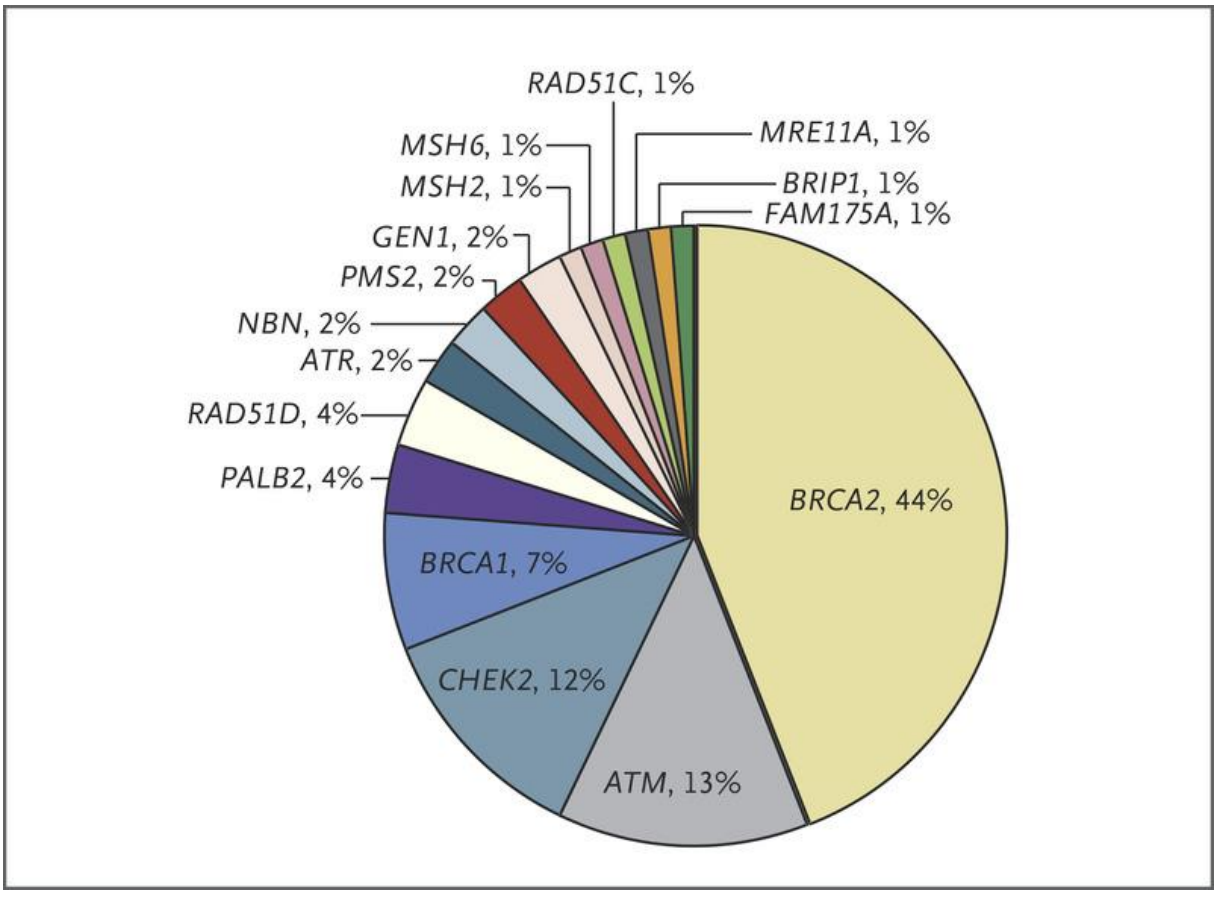


Figure 91: distribution of pathogenic germeline mutation

BRCA2: At 44%, it represents the most common mutation, highlighting its predominant role in cancer, particularly breast and prostate cancer. CHEK2: At 12%, this gene is also significant,

and involved in the response to DNA damage. TMJ: With 13%, it also plays a key role in regulating responses to DNA damage. BRCA1: at 7%, this gene is linked to an increased risk of breast and ovarian cancers. The other genes, such as PALB2 (4%), RAD51D (4%), and several genes at 2% or less (such as GEN1, PMS2, NBN, ATR, MSH2, MSH6, RAD51C, FAM175A, BRIP1, MRE11A), indicate less frequent mutations but still relevant in the context of cancer.

Other genes are involved in the specific stages of prostate cancer development (Figure). The progression of the latter presents a sequence of events beginning with initiation, where factors such as inflammation, oxidative DNA damage, and telomere shortening contribute to the transformation of normal cells into abnormal cells, leading to prostate intraepithelial neoplasia (PIN). This stage progresses towards the development of adenocarcinomas, first latent and then clinically detectable, with possible metastases. Key genes associated with these stages include NKX3.1 under regulation, MYC overexpression, and TMPRSS2-ERG fusion at initiation, followed by PTEN inactivation and ERK/MAPK pathway activation at progression, as well as EZH2 overexpression. Together, these processes and genes illustrate the complexity of prostate cancer's evolution, shedding light on the mechanisms behind its development and progression.

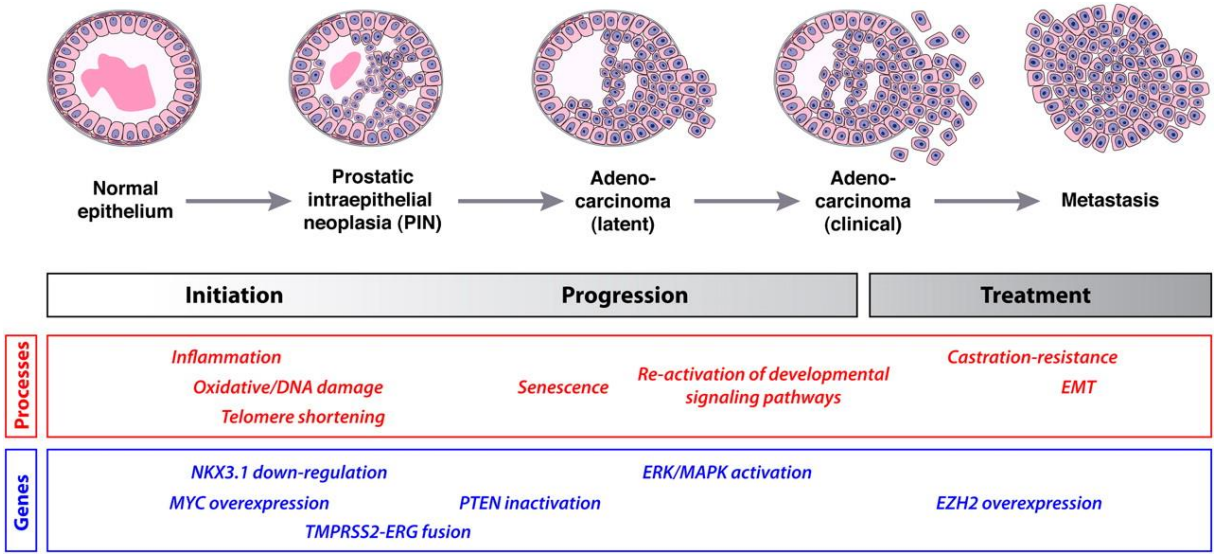


Figure 92: Prostate Cancer Progression: Mechanisms and Genes Involved

10 TUMOR MARKERS

Tumor markers are proteins found in the blood, tissues, or urine, which signal a malignant process. Some are specific to a particular cancer, others are more general and are increased in different types of cancers.

Tumour markers can be produced directly by a tumour or by other tissues in response to a tumour and can be metabolic products, acute-phase proteins, enzymes, hormones or antigens associated with tumours, such as carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP). Used in conjunction with X-rays and other tests, the detection of tumour markers in the blood can be very useful in detecting and diagnosing cancers. Although measurement alone is not sufficient to make a diagnosis, their detection may be important for the detection of some cancers at early stages, for monitoring cancer progression, choice and response to treatment, and for prognosis and estimation of severity.

Tumor markers have specific characteristics that make them useful for screening, diagnosing and monitoring cancers. Key features include:

1. Specificity: A specific tumor marker is associated primarily with a type of cancer or a specific location of the tumor. For example, CA 19-9 is often linked to pancreatic cancer, while PSA is a more specific marker of prostate cancer. However, this specificity is not always absolute, and some markers may be elevated in benign diseases.

2. Sensitivity: The sensitivity of a tumor marker refers to its ability to detect the presence of cancer in its early stages. A sensitive tumor marker is able to spot cancer cells even at an early stage, although high sensitivity can sometimes lead to false positives (when the marker is elevated in the absence of cancer).

3. Relationship to tumor burden: The concentration of a tumor marker in the blood may be correlated with tumor mass or progression. High levels of certain markers may reflect a significant tumour burden, which is often used to assess the course of a disease. For example, an increase in CA125 may indicate progression in ovarian cancer.

4. Usefulness in therapeutic monitoring: Certain tumor markers make it possible to monitor the effectiveness of a treatment. Their levels often decrease in response to effective therapy (surgery, chemotherapy, radiotherapy) and can increase if the disease recururs or progresses.

5. Possibility of dosing in biological fluids: Most tumor markers can be measured in biological fluids, including blood, but also sometimes in urine or other body fluids. This facilitates their use in the clinic for minimally invasive and repeated tests.

6. Non-specificity for certain markers: Some tumor markers, such as CEA (carcinoembryonic antigen), can be elevated in several types of cancer (colon, lungs, etc.) as well as in certain non-cancerous pathologies (chronic inflammatory diseases, cirrhosis, etc.). This is why they are used in addition to other examinations to avoid diagnostic errors.

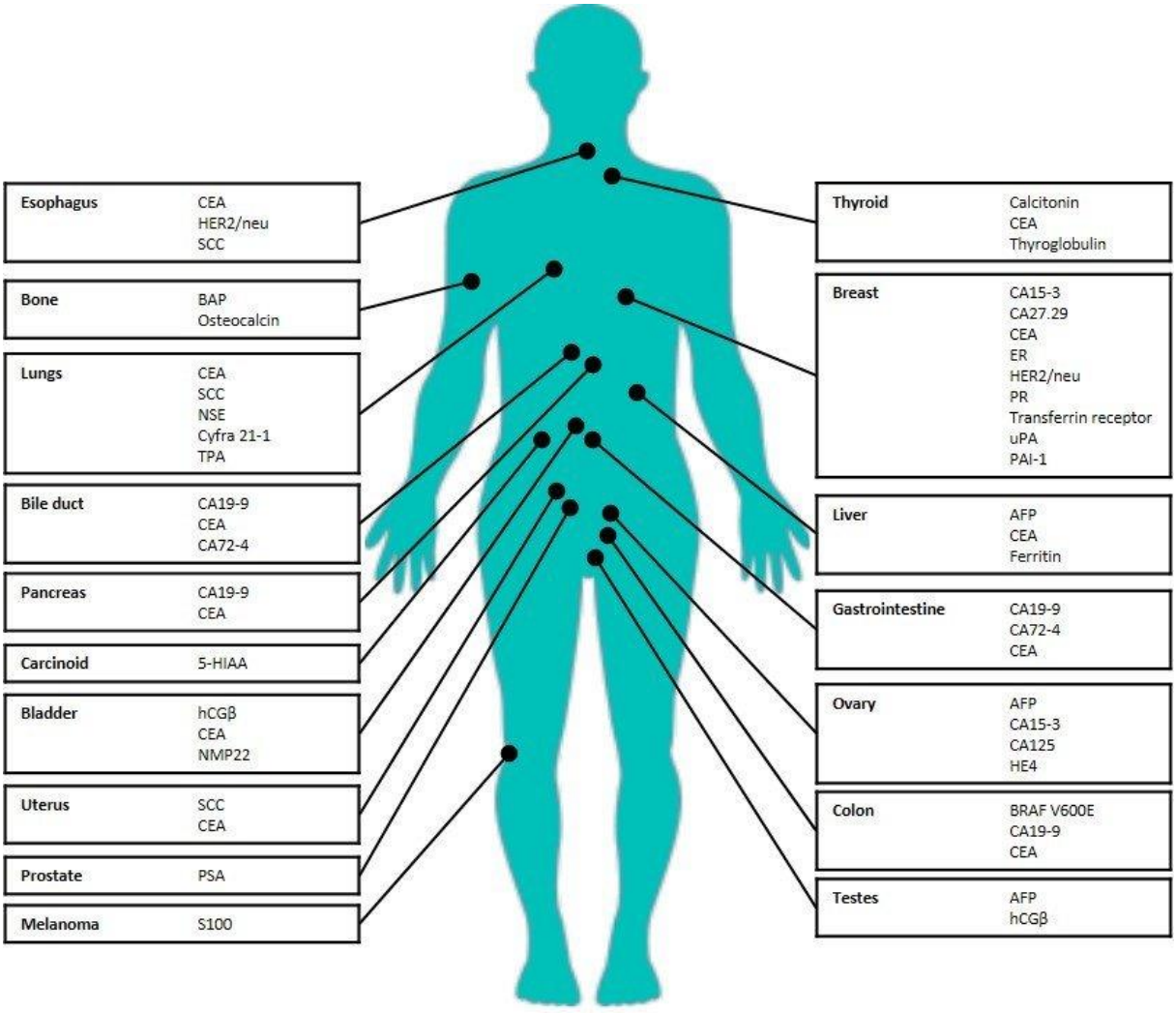


Figure 93: Tumoral marker (PSA: prostate-specific antigen; CA: cancer antigen; CEA: carcinoembryonic antigen; α-FP: alpha-fetoprotein; β-HCG: chorionic gonadotropic beta-hormone).

Tumor markers are valuable tools for cancer management, but their interpretation must be cautious and often integrated with other clinical data to avoid incorrect diagnoses.

11 CANCER STEM CELLS

Cancer stem cells (CSCs) are cancer cells (found in so-called "solid" tumors or hematologic cancers) that possess characteristics associated with normal stem cells, including the ability to give rise to the different cell populations present in a particular tumor. These cancer stem cells have been identified and isolated from a large number of cancers including the brain, colon and prostate. A new theory of cancer has recently taken hold in the scientific community. According to the latter, cancers develop from a very specific subpopulation of cancer cells, called "cancer stem cells" (CSCs).

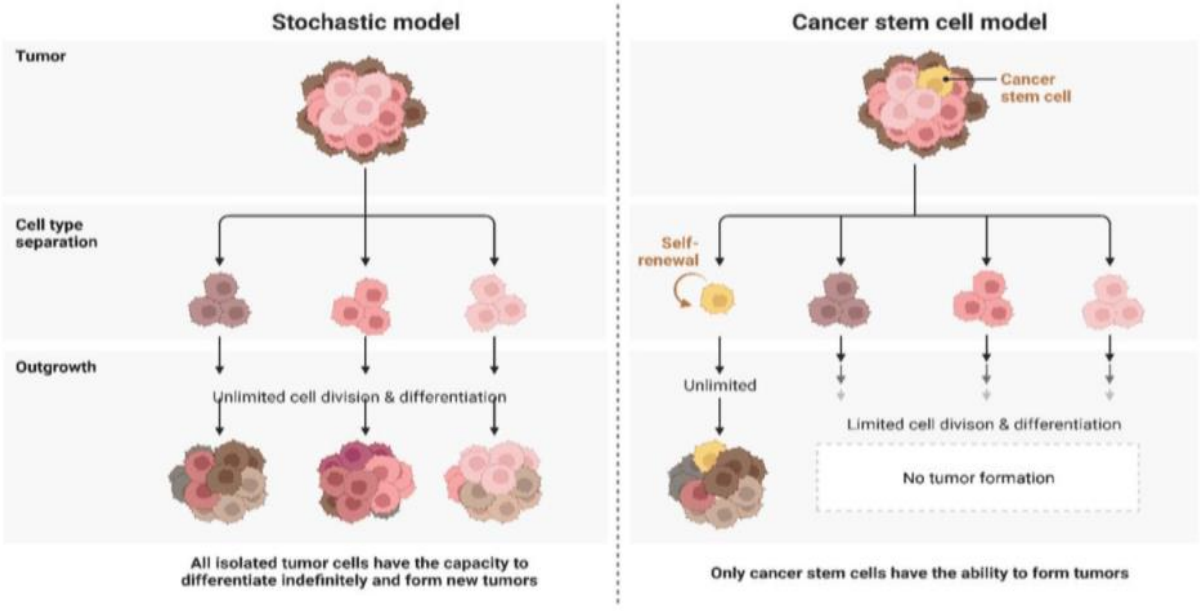


Figure 94: Schematic representation of the stochastic and CSC models of carcinogenesis

Proponents of the CSC theory argue that relapses are caused by these cells, which are more likely to evade conventional therapies. As a result, they argue that the elimination of all SCCs, in a given cancer, is necessary and sufficient to cure the patient.

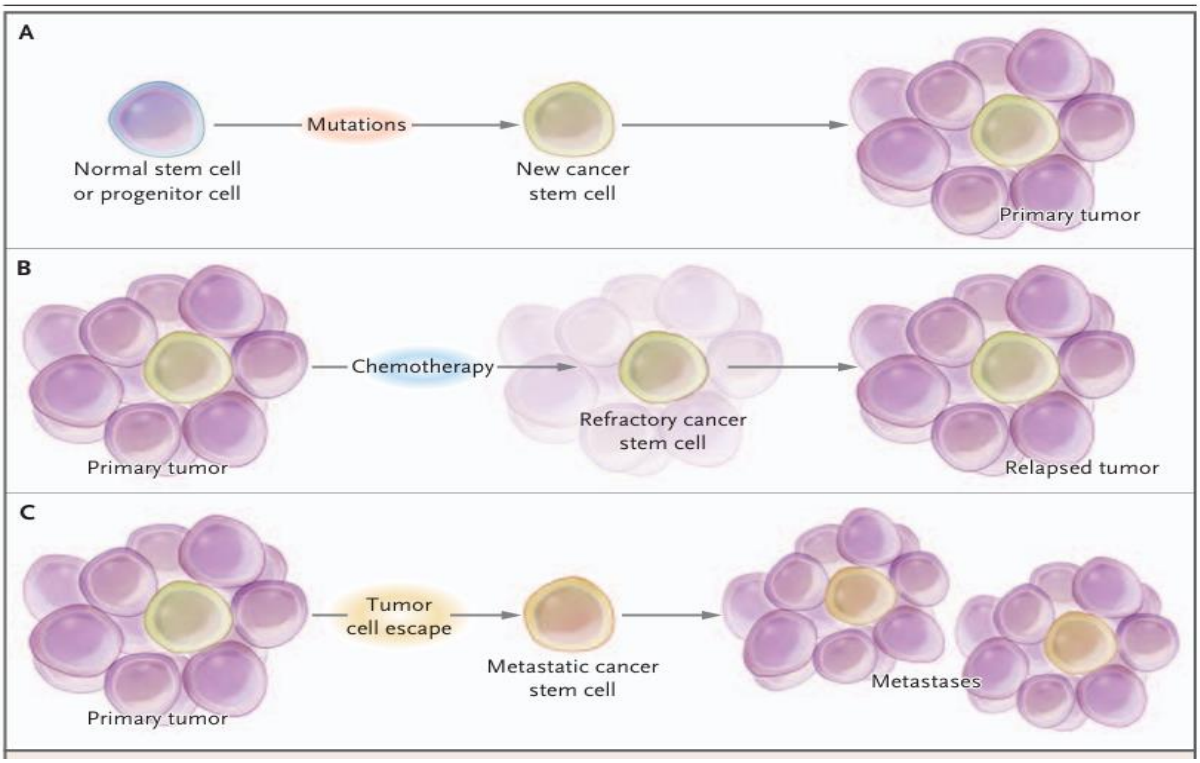


Figure 95 : Scenarios Involving Cancer Stem Cells. For tumors in which cancer stem cells play a role, at least three scenarios are possible. First, mutation of a normal stem cell or progenitor cell may create a cancer stem cell, which will then generate a primary tumor (Panel A). Sec ond, during treatment with chemotherapy, the majority of cells in a primary tumor may be destroyed, but if the can cer stem cells are not eradicated, the tumor may regrow and cause a relapse (Panel B). Third, cancer stem cells aris ing from a primary tumor may emigrate to distal sites and create metastatic lesions (Panel C).

Cancer stem cells (CSCs) play a crucial role in resistance to cancer treatments. Cancer stem cells have inherited or diverted from normal stem cells multiple and complementary defense strategies allowing them to resist chemotherapy: (1) a protective hypoxic "niche" that is not very accessible to cancer drugs and promotes the quiescence of cancer stem cells, (2) the expression of membrane efflux transporters and (3) enzymes of detoxification metabolism in order to block the activity of anticancer drugs, (4) an increased capacity for DNA control and repair and (5) catabolism of reactive oxygen species in order to block the cellular effects induced by chemotherapy, and finally (6) an increased capacity for resistance to programmed cell death through the expression of anti-apoptotic genes.

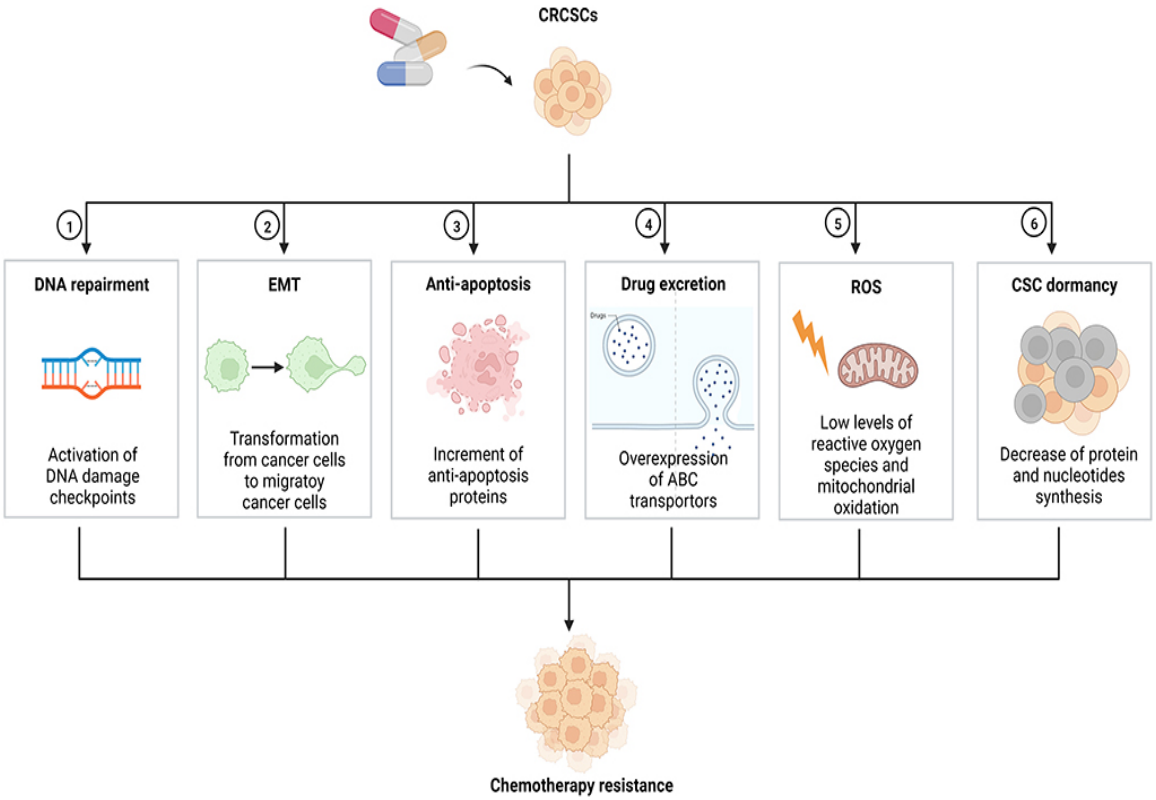


Figure 96: Mechanisms on chemotherapy resistance of CRCSCs (colorectal cancer stem cells).

ABC (ATP-binding cassette)

12 CANCER THERAPIES

Cancer therapies are all treatments used to treat cancers. In general; They are represented by surgery, radiotherapy, chemotherapy, immunotherapy, hormone therapy and targeted therapy. These protocols can be used alone or in combination depending on the type and stage of the cancer.

12.1 Surgery

It most often consists of the removal of the cancerous tumor as well as any surrounding tissue that may contain cancer cells. Often used as an initial treatment for *tumors in situ* and supplemented with chemotherapy, radiotherapy, and hormone therapy.

12.2 Radiotherapy

A physical treatment that uses ionizing radiation to eliminate cancer cells while preserving healthy tissue as much as possible. It can be used alone or in combination with surgery or chemotherapy.

12.3 Chemotherapy

It is a chemical therapy that involves targeting the division not only of cancer cells but also of normal, fast-dividing cells such as those in the bone marrow, intestinal mucosa, and hair follicles, thus leading to their death. This process therefore causes side effects such as hair loss; nausea and decreased blood cell counts

It can be subdivided according to their target: those that target DNA such as alkylating agents and intercalating agents. those that target enzymes such as antimetabolites and antitopoisomerases. Those that target the cytoskeleton as well as the poisons of the mitotic spindle.

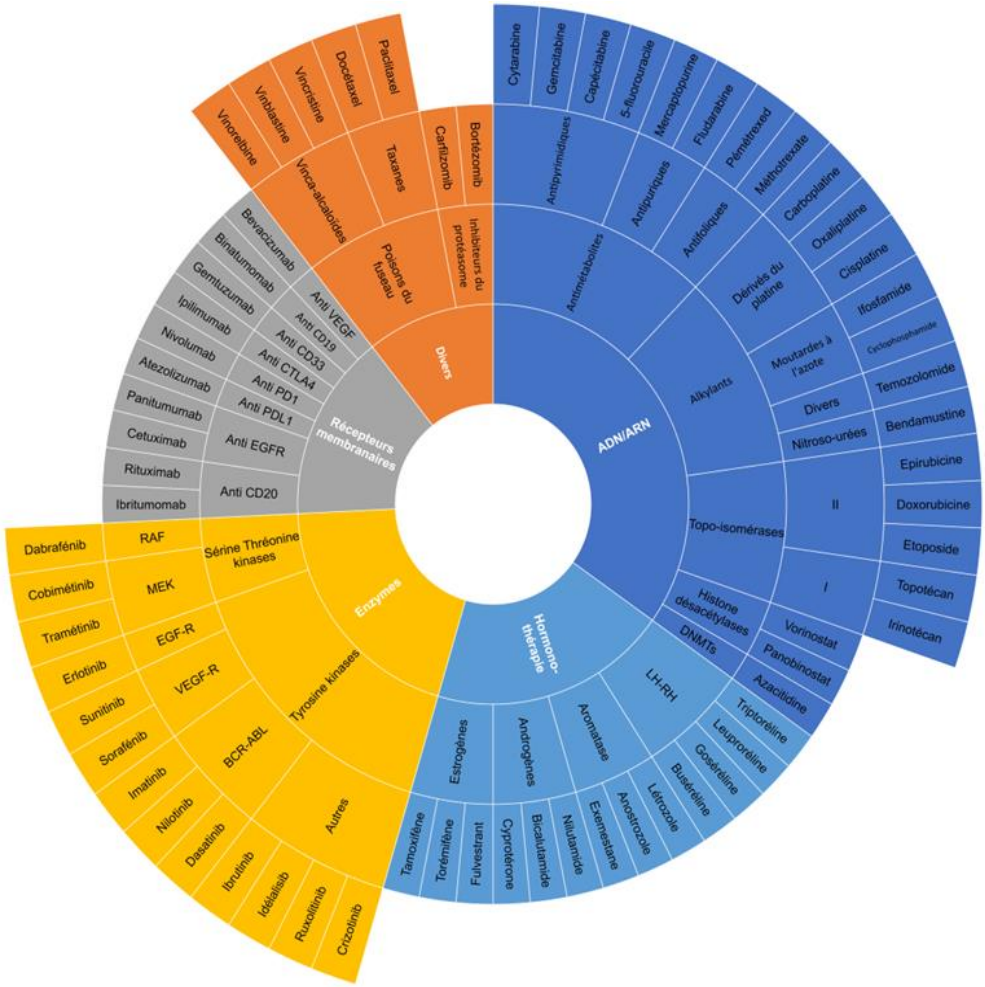


Figure 97 : Target and mechanism of action of anticancer drugs

12.4Immunotherapy

Immunotherapy works by stimulating the anti-tumor immune system. If the anti-tumor immune response is insufficient to eliminate the tumor, immunotherapy aims to increase the response. Different techniques exist to meet these objectives: passive immunotherapy, which consists of administering molecules or cells of the immune system to increase the pre-existing immune response (e.g., monoclonal antibodies, CAR-T cells), and active immunotherapy, which corresponds to the establishment, or stimulation, of the patient's antitumor immune response (e.g., cytokines such as interferons), vaccination).

12.4.1Monoclonal antibodies

monoclonal antibodies can either **target the cancer cell** such as antiCD20 antibodies (rituximab) (a marker expressed by B cells targeted in certain lymphomas) or anti-HER2 antibodies targeted in breast cancer, or **be immunomodulatory** (target and inhibit immune

system checkpoints) (figure) such as the anti-CTLA-4 monoclonal antibody (a molecule that blocks the proliferation of T cells) (ipilimumab) which blocks this molecule and thus induces the multiplication of T lymphocytes or the anti-PD-1 antibody (pembrolizumab, or nivolumab) which have been developed to inhibit the activity of PD-1 (In the case of cancer, tumor cells express its PD-L1 ligand. The PD1/PD-L1 interaction results in T-cell apoptosis and prevents lymphocyte apoptosis.

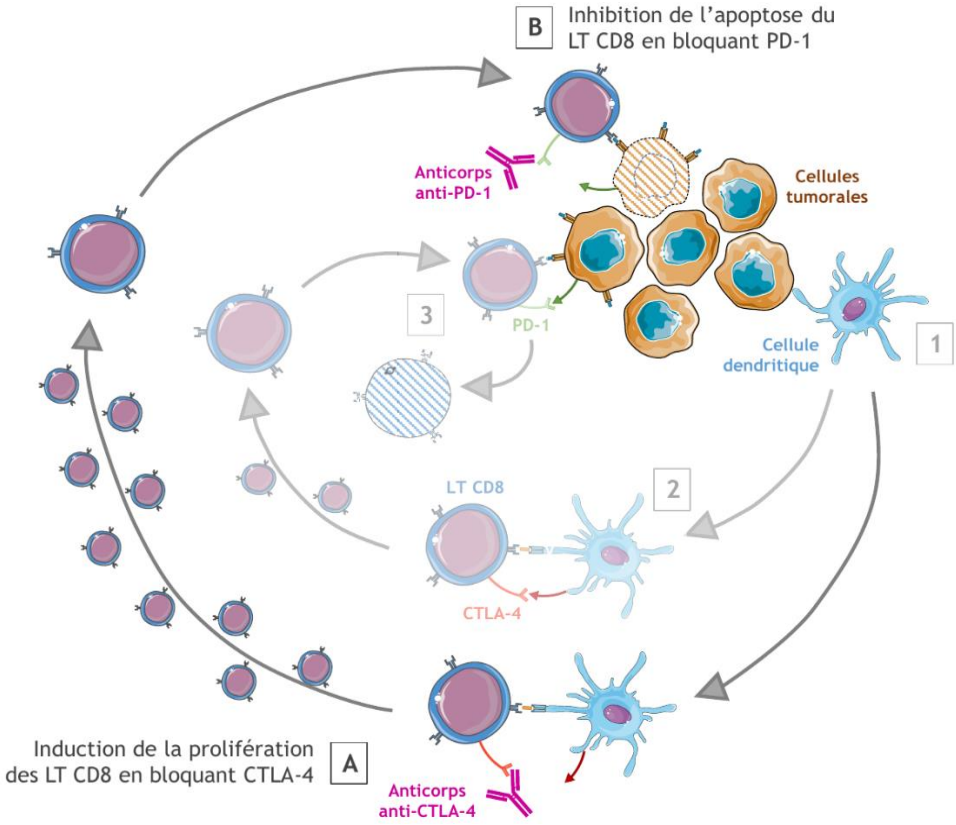


Figure 98: Action of immunomodulatory monoclonal antibodies

12.4.2 Therapeutic vaccines

Therapeutic vaccines to treat cancer aim to strengthen the specific immune response against tumor cells. Unlike preventive vaccines, which protect against infection, therapeutic vaccines are designed to treat patients who already have cancer by stimulating the immune system to recognize and attack cancer cells.

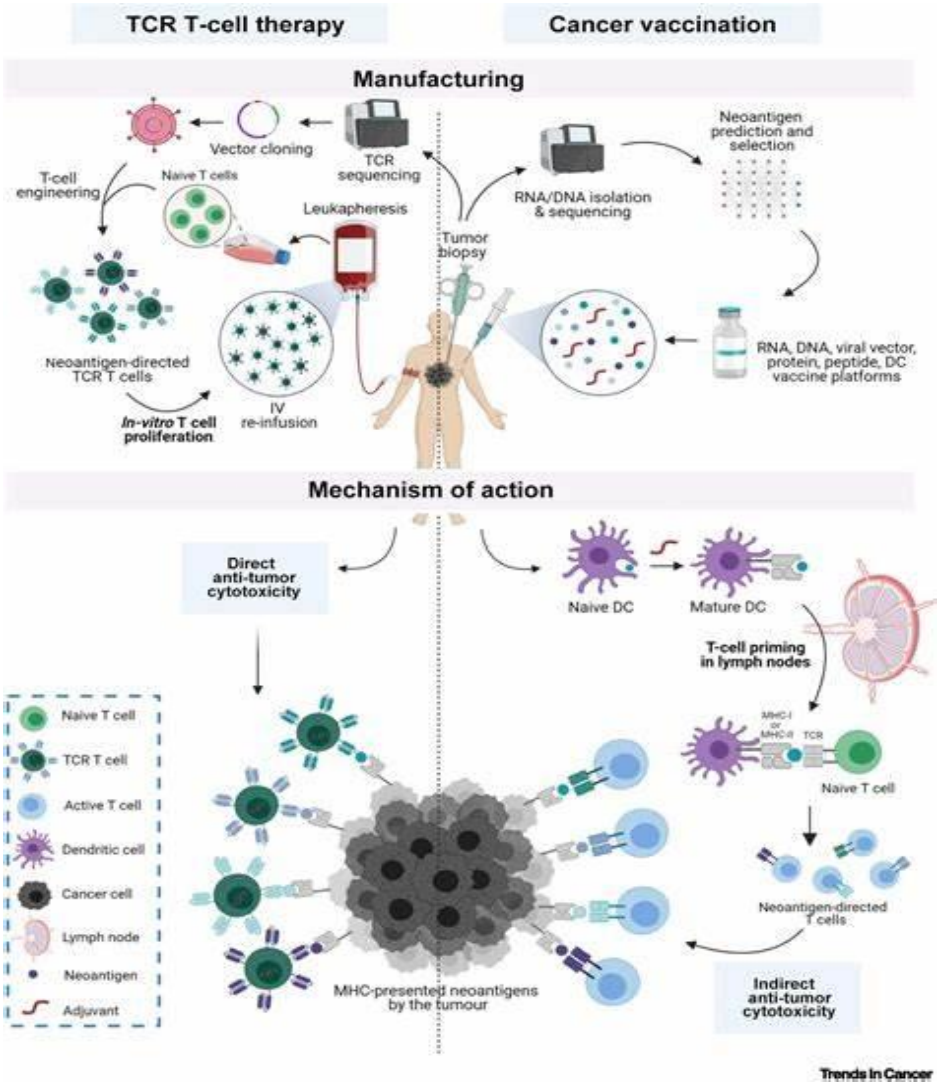


Figure 99 : therapeutic vaccin

12.5 Cell therapy: the example of CAR-T cells

Cell therapy with chimeric antigen receptor (CAR-T) cells is a significant advance in cancer treatment. This innovative approach involves taking T cells from the patient, which are then modified in the laboratory to express a chimeric receptor specific to a tumor antigen.

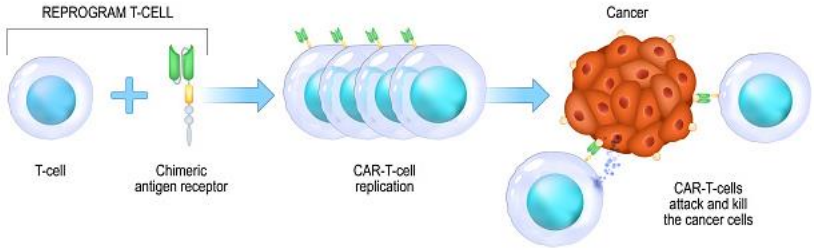


Figure 100 : immunotherapy based on CAR-T cells

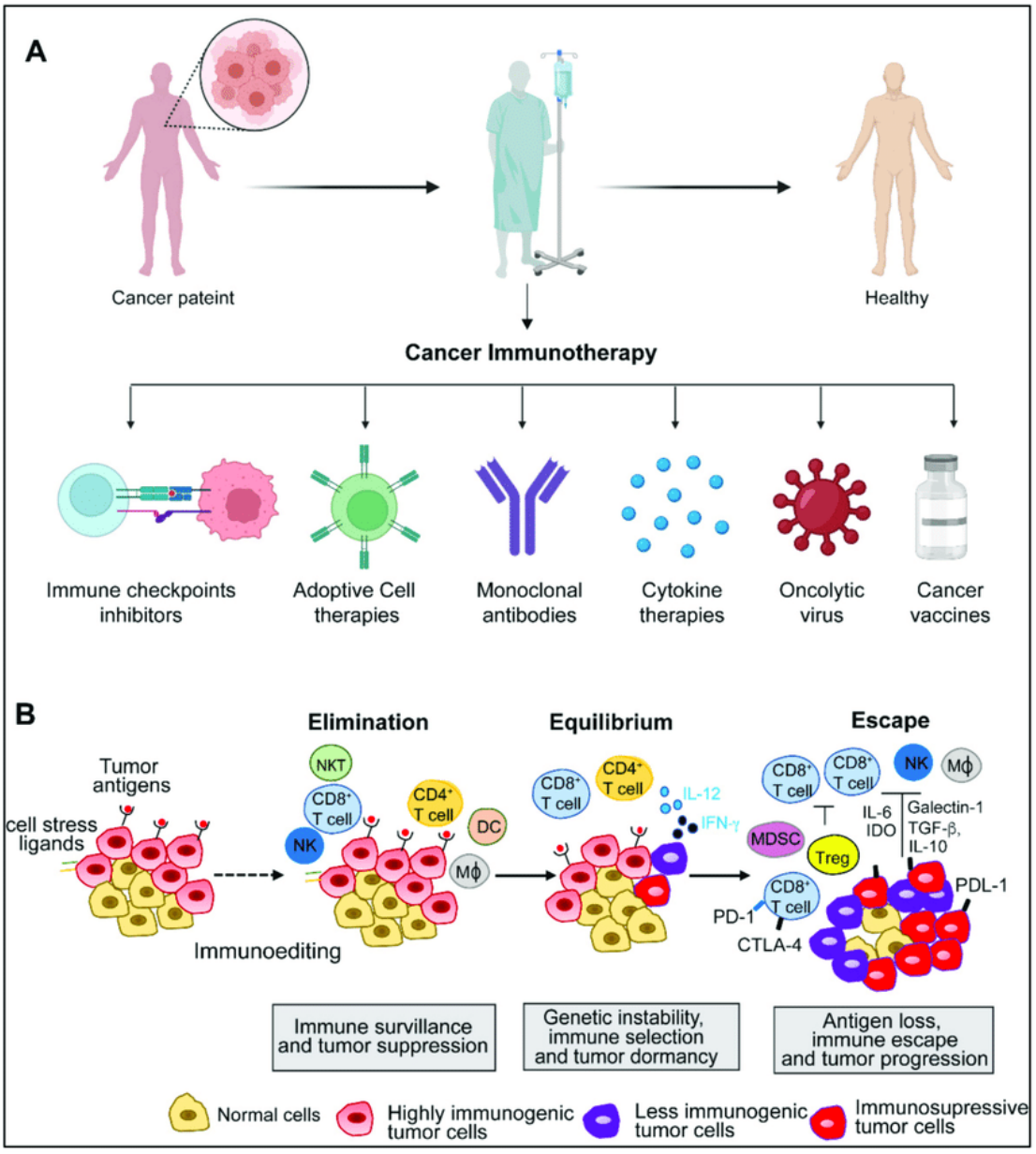


Figure 101: Mechanisms and Approaches to Cancer Immunotherapy: A: Immunotherapy strategies and their impact on the patient's treatment pathway. B: Phases of the immune response: Elimination, Balance and Evasion in tumor immuno-editing.

12.6Targeted therapy

Treatment is directed against a cellular target of cancer cells (overexpressed receptor such as ER, VEGFR or HER2, specific ligand such as VEGF or EGF with the aim of specifically stopping the growth of tumor cells or angiogenesis. The different targets are illustrated in the figure.

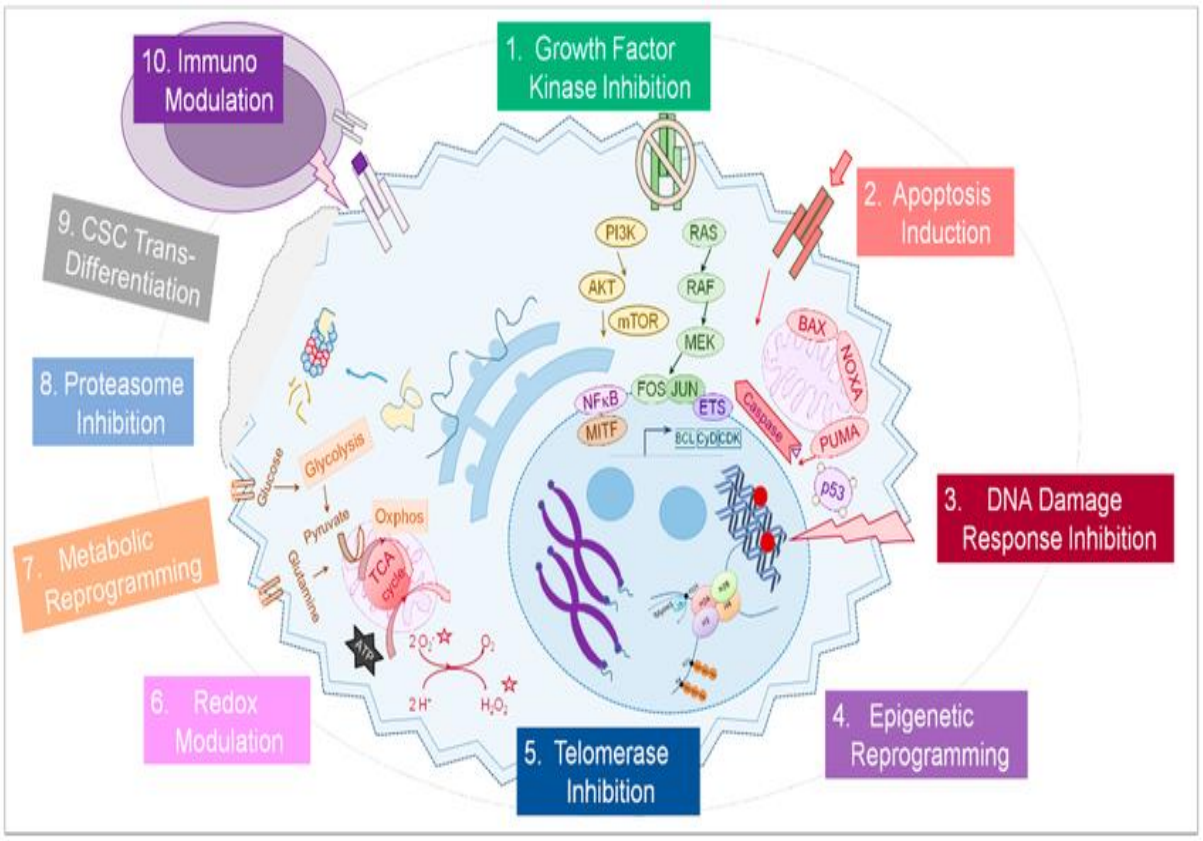


Figure 102 :The principles of targeted cancer therapy.

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