

On the origin of Carcinoma

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The origin of cancer remains one of the most important enigmas in modern biology. The prevailing paradigm has failed to grasp a comprehensive view of the disease. Naturally, therapies developed under the current assumptions are inadequate and cancer is practically an incurable disease. Meanwhile, descriptive studies continuously extend the molecular complexity of cancer without an equivalent advancement in its understanding. Furthermore, they tend to accumulate inconsistencies inexplicable under the classical view. This paper presents a compelling theory of the origin of carcinomas. By hypothesis, a series of generic events in epithelial tissues promoted by cellular aging and inflammation enables the reactivation of developmental programs. The origin of carcinomas *in vivo* is described as the time-ordered cell state transitions undergone by epithelial cells in the hyperplasia due to replicative senescence and inflammation towards a mesenchymal undifferentiated endogenous cell state with cancerous behavior. In support of the theory, the molecular, cellular, and histopathological evidence is critically reviewed. A plausible model for the origin of carcinomas is presented to explain the mechanism underlying carcinogenesis from an evolutive and developmental perspective. The implications of the hypothesis in the current strategies for cancer prevention and treatment are discussed along with rational alternatives and some predictions for possible experimental validation.

Keywords: Senescence, Immortalization, Epithelial to Mesenchymal Transition, Carcinogenesis, Hypothesis

Introduction

Cancer is one of the leading causes of death worldwide. Every year 14 million people are diagnosed and 9 million will die from cancer. Despite the enormous amount of research and financial support during the last seven decades, no significant differences have been observed in mortality rates ¹. Late diagnosis has been considered the main challenge in oncology since therapy is ineffective in advanced stages. Currently, over half of the patients are diagnosed with metastatic disease ² and 90% will experience therapy failure due to tumor resistance or unbearable side effects ³. The initial favorable response in the control of tumor growth is inexorably followed by the progression despite therapy in less than six months ⁴. Regarding toxicity, the mortality derived from cancer therapeutics itself may appear in 50% of the cases during the first month of application ⁵. The side effects are responsible for 20% of the suspension of the protocol since patients could experience intolerable mucositis, myelosuppression, pulmonary fibrosis, cardiotoxicity, hepatotoxicity, and nephrotoxicity ⁶. Strikingly, most antineoplastic agents and radiotherapy induce cancer in animals and humans, including the oncologic patients in which they are called secondary cancers ⁷. Despite over 50 years of research since the original protocols against cancer, the overall contribution of chemoradiation to survival is less than 2% ⁸ and there is no convincing evidence that the newer and expensive agents outperform his predecessors ^{9, 10}. In essence, the lack of understanding of cancer itself, how it develops, and progresses continues to halt the development of effective therapeutic strategies ^{11, 12}.

The prevailing paradigm for the origin of cancer is the somatic mutation theory. From this perspective, cancer is a genetic disease in which DNA mutations lead to a cancerous phenotype¹³. Despite the lack of success, the rationale of therapy has been to kill cancer cells mostly with DNA poisons in combination with agents that target the molecular alterations conferred by the acquired genetic traits⁸. According to the somatic mutation theory the genetic alterations allow cancer cells to proliferate uncontrollably, the ability to invade nearby and distant tissues, thus compromising systemic functions that eventually cause death. The processes that are considered altered in cancer cells are the proliferation signals, the evasion of growth suppression, the resistance of cell death, the restoration of limitless replicative capacity, changes in energy metabolism, the activation of invasion and migration. Furthermore, it assumes that cancer cells are constantly evolving due to genomic instability and by a Darwinian mechanism cells become resistant to therapy and able to escape the immune response. Additionally, mutations somehow confer tumors the capacity to reshape the tumor microenvironment to support its progression by the reactivation of processes such as angiogenesis and inflammation¹⁴.

Despite the massive resources devoted to genomic sequencing of human cancers, in essence, it has not been possible to find an underlying genetic cause for carcinogenesis. Most oncogenes and tumor suppressors play crucial roles in the normal biology of cells¹⁵. The somatic mutation theory is also challenged by several experimental inconsistencies, for example, many carcinogens lack from mutagenic effects¹², cancer cells show morphological and transcriptional convergence regardless of the initial cellular phenotype¹⁶, and cancerous behavior can be adopted through processes of transdifferentiation¹⁷. Notably, the reversion of the malignant phenotype is possible through the induction of differentiation using chemical agents¹⁸, vitamins¹⁹, transcription factors²⁰, and interactions with the extracellular matrix or the stroma²¹. Those findings are incomprehensible with the current paradigm, and at the same time suggest an endogenous cellular state that can be accessed or discarded according to the nature of the stimulus.

In this paper, an alternative framework to understand the emergence and progression of carcinomas is proposed. In this hypothesis, it is considered that the functional, molecular, and developmental properties of epithelial cells, together with their organization as tissue promote their transformation. In addition, carcinomas share a pattern of histological progression and molecular events that suggest a generic process underlying carcinogenesis. Hence, one of the premises is that cancer is organized by conserved mechanisms operating behind the genetic alterations. It is proposed that hyperplasias enable the reactivation of developmental processes and that malignant cells arise from cellular senescence using time-ordered cell state transitions. In support of the idea, is provided a detailed overview of the molecular, cellular, and structural process that allows the reiteration of embryonic programs in epithelial hyperplasia. To accomplish an integrated hypothesis for the origin of carcinomas, this paper challenges the granted role of mutations, senescence, inflammation, fibroblasts, stem cells, and the epithelial to mesenchymal transition in carcinogenesis. Instead, the plasticity of epithelial cells induced by aging, inflammation, and tissue rearrangements are considered the most important factors for *in vivo* carcinogenesis. Cancer as the pathological outcome of the same mechanisms activated in development and during tissue regeneration.

The paper is organized into eight parts. The first presents the intrinsic plasticity of epithelial cells, the progressive nature of carcinomas, and the conserved histological and molecular events during their carcinogenesis. The second part covers the current role of senescent cells in carcinogenesis and their susceptibility to transformation. The third part focuses on the epithelial to mesenchymal transition and the mirroring of epithelial carcinogenesis with the spontaneous immortalization of epithelial cells *in vitro*. Part fourth presents the theory of the origin of carcinoma and the impact of aging, inflammation, and structural factors in tissues and their implication on the nature of metastasis. Part five covers aspects related to inflammation and cellular plasticity. Section six is devoted to uncovering the elusive nature of myofibroblasts. In part seven is discussed stemness as the cellular state with malignant behavior and cancer and chronic

diseases as part of development. The last section discusses the current strategies of cancer prevention and therapy in the light of the hypothesis and suggests a rational alternative and some predictions for possible experimental validation.

1. The epithelial cells and the transformation of the epithelium

1.1. The intrinsic plasticity of epithelial cells

Cancer that originates from epithelial tissue is called a carcinoma and is the most common type of neoplasia in humans. Up to 85% of cancer diagnosed in adults begins in the skin, breast, endometrium, prostate, colon, lung, pancreas, bladder, liver, or cervix ²². In general, epithelial tissues are constituted by two types of cells, the epithelial cells that line the surface of a tissue or organ and the mesenchymal cells located in the extracellular matrix. At the microscopic level, they are composed of epithelial cells attached to the basement membrane, which establishes an axis of apical-basal polarity and they communicate with each other through cell-cell interactions. Below the basement membrane is the stroma, which is formed by the three-dimensional extracellular matrix synthesized by the mesenchymal cells. Broadly, epithelia form specialized three-dimensional structures adapted to their physiological function, as is the case with secretory organs. These tissues secrete glandular products mainly from the apical surface and some also from the basal surface. Epithelial cells also have a polarized distribution of organelles with distinct apical and basolateral domains. Transmembrane proteins have cytoplasmic tails that bind to elements of the cytoskeleton, forming protein complexes necessary for their organization. These domains allow the polarity of the plasma membrane to be established, they provide an adhesion site for intercellular junctions and anchoring for the molecules of the intracellular signaling pathways responsible for the differentiation and survival of epithelial cells ²³.

Transcription factors with a conserved ETS domain (epithelium-specific) are considered specific to epithelia and play a crucial role in their differentiation. This family of proteins has 26 members, which are important mediators in the morphogenesis and development of epithelial tissues by regulating their gene expression. The conserved ETS domain allows direct binding to promoter and enhancer regions of target genes crucial for the proliferation and formation of the cellular interactions of epithelial cells. The studies of mice in which the expression of ETS proteins was genetically eliminated revealed their importance in the correct differentiation of the structures of epithelial tissues ²⁴. Some members of the family have been identified ubiquitously expressed, however, a subgroup called ESE proteins (ETS specific of epithelium) are reserved for epithelial tissues ²⁵. These transcription factors are ESE-1, ESE-2, ESE-3, and the PDEF (Prostate-derived Ets factor). The transcription factor ESE-1 is widely expressed in organs such as the lung, stomach, kidneys, colon, and skin ^{26, 27}. ESE-3 is constitutively expressed in the lung epithelium, prostate, pancreas, salivary glands, and trachea ²⁸. PDEF is specifically expressed in the glandular epithelial cells of the prostate ²⁹. Finally, the transcription factor ESE-2 is expressed from the extra-embryonic ectodermal lineage, it is essential for the survival of the embryo and in the formation of the placenta in mammals. This protein is expressed in the mammary gland, salivary gland, kidney, stomach, and skin ³⁰. Consistently, it has been found that the silencing or downregulation of the ESE family of proteins is necessary for the loss of differentiation of the epithelial phenotype and the generation of carcinomas ³¹.

Epithelial cells are terminally differentiated; however, they maintain great cellular plasticity that is evident since the early stages of development. This intrinsic plasticity could be the origin of the susceptibility of epithelial tissues to malignant transformation. Interestingly, the first type of differentiated tissue to emerge during embryo development is the epithelia. From the eight-cell stage until the blastula, the blastomeres adhere using the binding protein epithelial cadherin (E-cadherin) and syndecan. This allows the aggregation of cells and the formation of a two-dimensional sheet. Differentiation of the epithelium is further promoted by the interaction of receptors with components of the basal lamina. The second phenotype in appearing is

mesenchymal, it arises from epithelial cells that undergo the process of epithelial to mesenchymal transition (EMT). The mesenchymal phenotype basically emerges from the suppression of the factors that induce epithelial differentiation²³. The increased expression of the zinc-finger binding transcription factor (Snail), the Snail Family Transcriptional Repressor 2 (Slug), the twist family bHLH transcription factor 1 (Twist1), the Twist-related protein 2 (Twist2), and the Zinc finger E-box-binding homeobox protein 1 (ZEB1) and ZEB2 promote the downregulation of the ESE proteins. As a result, the E-cadherin and the epithelial phenotype are substituted by the neural cadherin (N-cadherin), vimentin, and the rest of the mesenchymal biomarkers and the eventual full acquisition of a fibroblastic morphology³². The process of EMT begins in the gastrulation with the breakdown of the basement membrane that underly the epiblast and is associated with the effects of fibroblast growth factor (FGF) on cells of the primitive streak and with the activation of the mesenchymal transcription factors such as Snail. The induction of EMT is completed with additional signals that lead to the migration of cells within the primitive streak. These cells undergo the reverse process called the mesenchymal to epithelial transition (MET) and originate the endoderm or remain differentiated in the mesenchyme and create the mesoderm. This migration of mesenchymal cells through the embryo is essential for proper morphogenesis³³.

The intrinsic plasticity of epithelial cells displayed during embryo development resembles many of the observed phenomena in the carcinogenesis of epithelial tissues. Therefore, the events that result in the reactivation of such a process in the adult could be sufficient to initiate the malignant transformation of the epithelium and its dissemination, which outcome is the lethal metastatic disease.

1.2. The carcinogenesis of epithelial tissues

The incidence of carcinomas increases exponentially with age, especially in patients suffering from chronic inflammation. The relationship between aging, inflammation, and tumorigenesis is traditionally understood from the genetic point of view of cancer. Namely, aging and inflammation increase the chances of developing and accumulating random somatic mutations that eventually generate clones of epithelial cells with cancerous characteristics³⁴. In contrast, a thoughtful examination of the histological progression of carcinomas in adults suggests a robust pattern of cellular and molecular events that suggest developmental processes rather than events generated by chance.

Histology is the study of the anatomy of cells and tissues and remains as the gold standard for cancer diagnosis. No molecular studies have overcome the careful examination of tissues in the microscope. The appearance of the epithelial tissue determines whether is normal, hyperplastic, or cancerous and its degree of malignancy. The use of complementary techniques provides a deeper insight into the molecular events that are taking place in the tissue by the identification of biomarkers. In this regard, current histological data support the progressive nature of carcinomas³⁵⁻³⁷. From this perspective, carcinomas are preceded by atypical hyperplasia that in turn progress from typical hyperplasia. The presence of atypia increases five times the risk of developing carcinomas. Therefore, atypical hyperplasia is considered the precursor of carcinoma *in situ* (CIS), which in turn increases 10 times the relative risk of developing invasive carcinoma. The carcinoma arising from the CIS then progresses from low to high histological grade (G1-G2-G3). The latter is a classification that describes the cellular and nuclear characteristics of cells and stages its degree of differentiation³⁷. Furthermore, the histological progression of carcinomas in humans is also recapitulated in murine models of chemical carcinogenesis³⁸ (Fig. 1.).

It seems very unlikely that the temporal order of histological events during epithelial carcinogenesis is the random result of genetic mutations and Darwinian selection. Instead, the progressive transformation of epithelial tissues suits better the premises of cancer as a developmental disease. From this perspective, cancer emerges by the reactivation of embryonic programs and not from novel traits earned by mutagenesis³⁹.

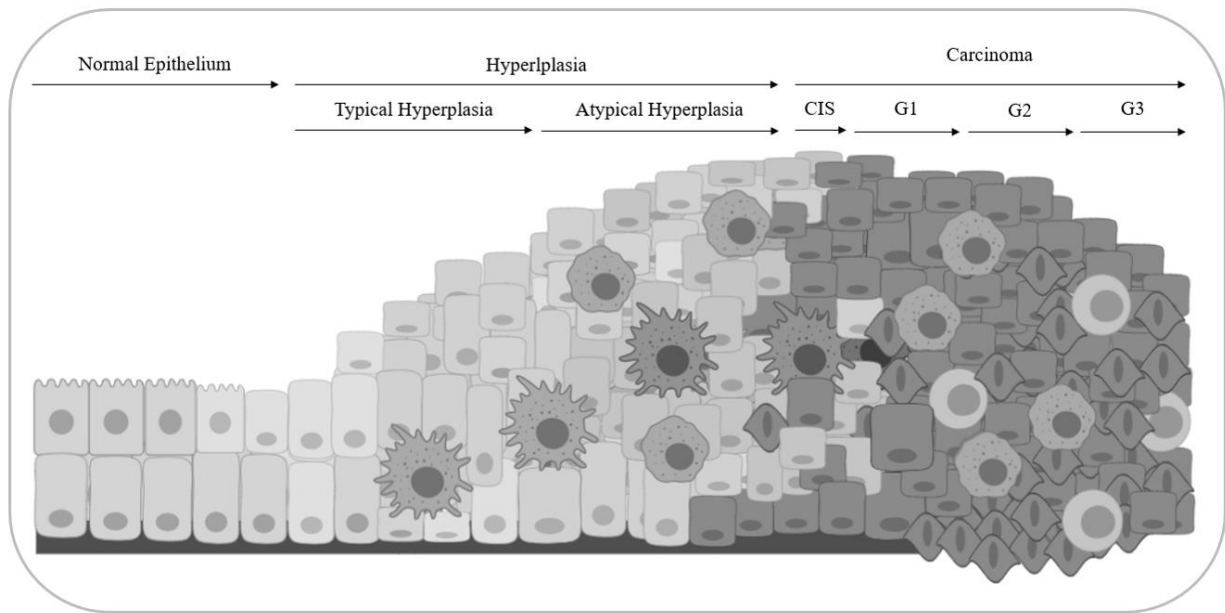


Fig. 1. The progressive nature of carcinomas.

Hyperplasia is considered the precursor lesion of cancer that originates in epithelial tissues. The diagnosis of atypical hyperplasias increases the risk of being subsequently diagnosed with invasive cancer ⁴⁰. In hyperplasias, are common the detection of biomarkers of cellular senescence such as short telomeres, expression of the protein p16, and the infiltration of immune cells ⁴¹⁻⁴³. As it progresses to carcinoma, the recovery in the length of the telomeres, telomerase enzyme activity is observed while decreasing the presence of senescent cells ^{44, 45}. In high-grade carcinomas, glandular histology and biomarkers of epithelial differentiation are lost ^{46, 47} together with an increase in the expression of transcription factors of a mesenchymal phenotype, the presence of metastases ⁴⁸, and stem cell biomarkers ⁴⁹.

The following section describes the molecular events that would trigger epithelial carcinogenesis during aging, the role of inflammation, and the process that shapes the histological progression towards the gradual loss of differentiation.

2. The aging of epithelial tissues

2.1. The hyperplasia stains positive for senescence

The hyperplasias of the epithelial tissue are characterized by the presence of senescent cells, but its progression to carcinomas is accompanied by their loss ^{41, 50}. Therefore, the changes that hyperplasia undergoes *in vivo* are crucial for the understanding of epithelial carcinogenesis. The presence of senescent cells increases with age, mainly in tissues that contain mitotically competent cells ⁵¹, which is consistent with the steady proliferation of epithelial tissues for their renewal and the maintenance of functions ⁵². However, inherent to the biochemistry of the replication of genetic material, telomeric DNA base pairs are lost in each cell cycle. Telomeres are DNA sequences that, together with associated proteins, cover and stabilize the ends of chromosomes and prevent them from degradation or fusion ⁵³. Eventually, short telomeres activate a signal of genetic damage that originate cell cycle arrest and the acquisition of the phenotype known as replicative cellular senescence ⁵⁴. Senescent cells coexist with short telomeres in hyperplasias and trace the mitotic rate of the affected epithelial tissue ⁵⁵.

The DNA damage response (DDR) is activated by telomere erosion ⁵⁶. In senescent cells are detected structures in the telomeres known as the DNA repair foci, that are also formed during double-strand breaks ⁵⁷. One of the initial steps in the formation of those compartments is the phosphorylation of the histone family member X (H2AX) by the ATM protein kinase (Ataxia telangiectasia mutated) ⁵⁸, which also activates the proteins p53 and Chk2 (Checkpoint kinase 2) ⁵⁹. In turn, p53 activates its transcriptional targets, such as the

cyclin-dependent kinase inhibitor 1 (p21)⁶⁰ and PML (Promyelocytic leukemia protein)⁶¹; both recognized biomarkers of senescence⁶². The p21 protein inhibits the activity of the cyclin-CDK 2/4 complexes and the family of transcription factors E2F leading to cell cycle arrest⁶³. The PML protein is an essential component of the nuclear bodies that also accumulate in the senescent cells⁶⁴. These compartments of proteins belong to the nuclear matrix, a superstructure related to DNA replication, transcription, and epigenetic silencing⁶⁵. PML recruits the proteins cyclin-dependent kinase inhibitor 2A (p16), p53, and the pRb / E2F complex to the nuclear bodies and modulates the expression of genes associated with cellular senescence⁶⁶. The gene p16 is located within the locus of the inhibitors of kinase (Ink4b/ARF/Ink4a) on chromosome 9p21. The locus encodes three genes; p16 and using an alternative reading frame (ARF) is enabled the transcription of the ARF protein product of CDKN2A (p14), and the cyclin-dependent kinase inhibitor 2B (p15)⁶⁷. The induction of senescence recruits the polycomb proteins (PcG), the Jumonji domain-containing protein 3 (Jmjd3), the enhancer of zeste homologue 2 (EZH2), and the Mixed lineage leukemia 1 protein (MLL1) to the locus Ink4b/ARF/Ink4a⁶⁸. The dissociation of the PcG proteins and the activity of the histone demethylases and methyltransferases result in the reconfiguration of chromatin and the transcriptional capability of cells⁶⁹. On the other hand, the proteins p16 and p15 inhibit the activity of the cyclin-dependent kinase (CDK) 4 and 6 preventing both, the phosphorylation of Rb protein and the actions of the E2F⁷⁰. Therefore, in senescence, the reorganization of chromatin coexists with the cell-cycle arrest⁷¹. Besides, the p14 protein increases the activity of p53 by the negative regulation of its inhibitor the protein murine double minute 2 (Mdm2). Thus, in senescent cells, the cell cycle arrest is temporarily amplified⁷², but the epigenetic changes will have some paradoxical consequences⁷³.

The cellular senescence was positioned as an irreversible arrest in the cell cycle which function in cancer as a tumor suppressor. Hence, precancerous lesions should overcome senescence to restore proliferation⁷⁴ by mutations or deregulation in the p16 and p53 signaling pathways⁷⁵. Later, the description of the secretory phenotype of the senescent cells, not only reinforces the potential role of inflammation in the transformation of the aged tissue but also offers a suitable explanation to overcome the molecular restrictions imposed in the cell cycle. Namely, the increased production of the mediators of inflammation and reactive oxygen species would lead to an increased chance of mutations that results in the abrogation of cellular senescence. The secretion of senescent cells includes cytokines, chemokines, growth factors, and proteases⁷⁶ which its expression was found to be coordinated by the transcription nuclear factor kappa B (NF-κB)⁷⁷. This protein itself portrays the process of inflammation, cancer development, and progression. The constitutive activation of NF-κB is frequent in tumors and its effects include the induction of proliferation, migration, and the inhibition of apoptosis⁷⁸. As consequence, the influence of the secretions of senescence cells on inflammation, proliferation, and tissue microenvironment plays a bigger role in the current understanding of the association of cellular aging and cancer. The premises are that in the initial phases of carcinogenesis, the immune system destroys senescent cells but during aging their accumulation surpass the clearance and the burden of inflammation became carcinogenic⁷⁹.

2.2. Overcoming the assumed role of cellular senescence

In this paper, a new role of senescent cells in epithelial carcinogenesis is considered. By hypothesis, the origin of carcinoma is the result of cellular transitions from senescent cells. To fully embrace the proposal is necessary to consider the replicative senescence as a cellular phenotype, and to explore the concept of cellular transitions and their interplay with the histological context. It is hypothesized that hyperplasia is the most plausible scenario to foster cellular transitions *in vivo* and cells arising from such events of transdifferentiation are primed with cancerous behavior. Therefore, aging and inflammation induce a deregulated but still endogenous cellular plasticity, which is the ultimate culprit for epithelial carcinogenesis.

The boundaries imposed in the concept of cellular senescence in carcinogenesis has been surpassed consistently in cell cultures. The epithelial cells overcome senescence in the absence of mutations in the locus

Ink4b/ARF/Ink4a^{80,81}, and far from being irreversible⁸², the cellular state of senescence is now considered an unstable condition from which cells eventually emerge⁸³. Furthermore, cellular senescence can be viewed as a cell fate since any cell regardless of its original lineage activates the same conserved program. The senescence is accompanied by the loss of its initial cellular biomarkers, morphology, and transcriptome⁸⁴. Cells accumulate in the G1/G2 phase of the cell cycle despite nutrients and growth factors⁸⁵. The metabolism remains active, acquires a greater adhesion to the extracellular matrix, albeit with the loss cell-cell contacts. The cells became secretory and more resistant to apoptosis⁸⁶. The structural rearrangements in the chromatin of senescent cells are so significant that can be viewed by light microscopy. The variations in the nuclear lamina are accompanied by the loss of heterochromatin domains and the gain of heterochromatic foci⁸⁶. Further molecular studies showed that the epigenome of replicative senescent cells undergo significant hypomethylation, and the result is the loss of repression of thousands of genes including those associated with cancer development⁸⁶.

The preceding is considered as a plausible endogenous event of epigenetic reprogramming in the epithelial hyperplasia. Cell reprogramming refers to the induction of the loss of cell differentiation in somatic cells towards an embryonic stem cell phenotype. In this context, the process of the epithelial cell undergoing replicative exhaustion is viewed as the first cellular transition, which means a lineage conversion among different cell phenotypes. The switch into another cell type of a different lineage through genetic reprogramming is also called transdifferentiation. Under this view, the replicative senescence predisposes the epithelia to carcinogenesis by epigenetic events. Once in the senescent state, the cell is prone to further cellular transitions and the mechanism that links the abrogation of cellular senescence along with subsequent events of dedifferentiation and carcinogenesis associates with inflammation. Observations of the transformation of hyperplasias and cultures of epithelial cells provide evidence of this assumption.

3. The transformation of epithelial tissues

3.1. Beyond cellular senescence

The concept of immunosurveillance considers the elimination of cancer cells as one of the most important functions of the immune system. For this reason, the infiltration of leukocytes in neoplasms was explained as an attempt of our body to destroy premalignant cells. Paradoxically, several studies have been shown that the infiltration of hyperplasias is positively correlated with their histological progression towards invasive carcinomas⁸⁷⁻⁸⁹.

According to the essential thesis of this paper, the senescent cells *in vivo* undergo another cellular transition by the effect of the cytokines along with the structural changes fostered by the inflammation in the microenvironment of the hyperplasia. The infiltration of cells of the immune system into epithelial hyperplasias occurs due to the secretory phenotype of the senescent cells. The consequence is an inflammatory cellular response that increases the concentration of cytokines creating a positive feedback loop. Then, it is theorized that a certain threshold of inflammation is necessary before the induction of an EMT-like transition in senescent cells, the result is cells that arise with cancerous behavior (Fig. 2.).

The relationship of the EMT with the suppression of cellular senescence in epithelial cells has been discussed primarily in the context of cancer progression. Consistent with the suggested temporal order of cellular transitions, it has been documented in the cultures of cancer cell lines that undergo senescence the emergence of subpopulations of cells with high expression of the mesenchymal transcription factor Zeb. Furthermore, the induction of oncogene overexpression first induces the senescence of cells, and upon the ectopic overexpression of the mesenchymal transcription factor Twist, the cellular senescence is eliminated by the induction of EMT. The effect of many oncogenic signals on senescent cells is accompanied by EMT and the transformation from the flat cell phenotype to a fibroblast morphology and the upregulation of the mesenchymal biomarkers⁹⁰. The capacity of the soluble mediators of inflammation to induce EMT in cell lines derived from carcinoma has been reviewed extensively⁹¹. The cytokines tumor necrosis factor alpha

(TNF α), the transforming growth factor beta (TGF β), interleukin 1 beta (IL1 β), and the interleukin 6 (IL6) converge in the activation of the transcription factor NF- κ B⁹²⁻⁹⁴, which constant stimulation upregulates the expression of Snail, Slug, Twist, Zeb and the Forkhead Box C2 (FOXC2). In turn, the mesenchymal transcription factors repress the locus Ink4b/ARF/Ink4a and the ETS transcription factors⁹⁵⁻⁹⁸. The detection of biomarkers of EMT and inflammation correlates with poor clinical outcomes, and the connection of the mesenchymal proteins in surpassing senescence *in vivo* is supported by the inverse correlation of the biomarkers in carcinomas⁹⁹. It can be said that all the characteristics to become a cancer cell are enabled through a single process of transdifferentiation¹⁰⁰. Cells subjected to EMT acquire metastatic capacity, resistance to apoptosis, immune evasion, stem cell qualities, and resistance to anti-cancer therapies. Furthermore, the process of EMT can be induced also by changes in the extracellular matrix (ECM), such as mechanical forces, matrix metalloproteinases (MMPs), and cytoskeletal rearrangements, hypoxia, pH, chemotherapy; doxorubicin, gemcitabine, fluorouracil, bevacizumab, nivolumab and radiotherapy¹⁰¹. The epithelial tissue affected with infiltrated hyperplasia possesses favorable conditions for the induction of the EMT in senescent cells from which emerge cells primed for carcinogenesis. The same order of cellular transitions is observed in the spontaneous immortalization of epithelial cells, and suggest a principle, a generic pattern that may be recreated during epithelial carcinogenesis *in vivo*.

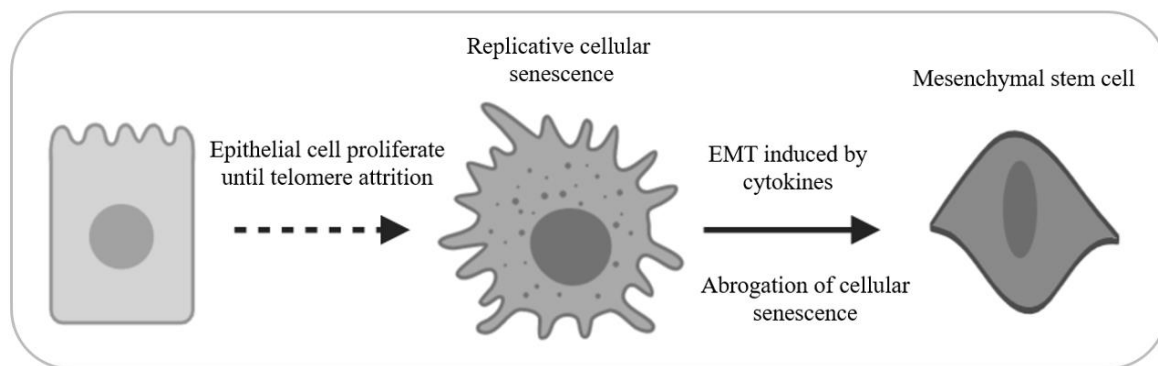


Fig. 2. Epithelial carcinogenesis is the consequence of cellular transitions.

The hypothesis considers that tissues exposed to cell proliferation eventually become enriched with senescent cells. The hyperplastic lesions will be infiltrated by immune cells that may induce EMT of senescent cells *in vivo*. The result is a cell that emerges with the ability to induce carcinomas. During the spontaneous immortalization of normal cells *in vitro*, the same series of cellular transitions is experienced by epithelial cells that arise from senescence with markers of basal lineage such as vimentin adopting a fibroblast morphology.

3.2. The order of cell immortalization

The study of primary cultures of normal human mammary epithelial cells has shown that cells can overcome senescence since the tenth doubling time. Those cells lose the expression of p16 and beta-galactosidase and resume proliferation. The overexpression of the proteins p53, p21, and the presence of chromosomal abnormalities are common findings. They include translocations, deletions, rearrangements, telomeric associations, polyploidy, and aneuploidy⁸⁰. Those reports also describe the gradual loss of epithelial markers since the 15-30 doubling time and the transformation of the luminal phenotype (epithelial) to a fibroblast morphology (basal or myoepithelial) with vimentin expression. The spontaneous immortalization over the long-term passages is understood as the consequence of genetic damage that result in the loss of p16, and telomerase reactivation¹⁰². Regardless of the cause, this phenomenon implies an especially illustrative connection between cellular senescence with EMT and supports the temporal order of cellular transitions suggested by the hypothesis (Fig. 2.). Thus, the spontaneous immortalization of epithelial cells *in vitro* may parallel the histological progression of carcinomas *in vivo*. The association of aging and inflammation with the increased chances for carcinoma development could be reduced to cellular transitions. However, as

outlined above the immortalization of normal epithelial cells, and the abrogation of cellular senescence by inflammatory cytokines culminates with the emergence of cells morphological and transcriptional more related to mesenchymal stem cells.

This hypothetical origin of carcinomas is incompatible with the notion that cancers from the epithelial tissue must correspond with epithelial cells that acquire malignant traits by mutations. Furthermore, the cancers that arise from mesenchymal tissues are sarcomas. To address these conceptual inconsistencies is necessary an overview of the staging and prognosis of carcinoma. The following demonstrates that the hypothesis is more compatible with the facts, although still controversial under current assumptions. For example, the five-year relative survival rate of most carcinomas depends on the stage at diagnosis. In the TNM staging system, this means a global stratification based on the size of the primary tumor, the affected lymph nodes, and the presence of metastases ¹⁰³. In the histological staging, the cancerous tissue is classified according to the degree of differentiation. Low-grade carcinomas show characteristics of normal tissue and tend to grow slowly. In contrast, high-grade cancers are poorly differentiated, lose the characteristics of normal epithelium, tend to grow faster, and respond poorly to therapy. Histologic grade is a prognostic factor for overall survival despite any TNM status. Essentially, in both systems, the probability of patients surviving more than five years exceeds ninety percent unless they already present metastasis or high grade at diagnosis ¹⁰⁴.

A notorious event during the progression towards a carcinoma of high grade is the inversion in the ratio epithelium to the stroma. In other words, the content of mesenchymal cells ¹⁰⁵ and the expression of their markers increase, such as vimentin, the alpha smooth muscle actin (α -SMA), N-cadherin, cadherin-11, and secreted protein acidic and rich in cysteine (SPARC) ¹⁰⁶, outnumbering the epithelial proteins. Additionally, the proportion of stromal content is an independent factor of poor prognosis ¹⁰⁷. Overall, it might be considered that both, the immortalization of normal epithelial cells *in vitro* and the lethality of carcinomas *in vivo* requires the adoption of mesenchymal traits.

3.3. The unavoidable fibroblasts contamination

Fibroblasts are considered the main effectors of fibrosis in normal or pathological conditions. The conventional view of fibrosis during epithelial carcinogenesis depicts that cancer-associated fibroblasts are cells programmed for the tumor to foster its progression ¹⁰⁸. These cells are the most abundant entity in the carcinoma microenvironment and compelling evidence demonstrated their abilities to promote all the so-called hallmarks of cancer ¹⁰⁹. In contrast to normal fibroblasts, they possess an increased proliferation rate and autocrine signaling. Over the years the presumed source of the cancer-associated fibroblasts has been evolving with observations that indicate an origin in the resident fibroblasts, pericytes, bone marrow, and more recently from epithelial cancer cells that undergo EMT *in vivo* ¹⁰⁹.

The investigation of the formation of fibrosis during inflammatory conditions clearly showed that normal epithelial cells can assume a mesenchymal phenotype and contribute to the generation of connective tissue associated with stromal proliferation and with scar formation ¹¹⁰. With the arrival of more sophisticated techniques, it was possible to gather conclusive evidence that not only does tumor epithelial cells *in vivo* give rise to cancer-associated fibroblasts but also that cells emerge with the expression profile of cancer stem cells ¹⁰⁹. The mesenchymal impurity of the carcinomas is an endless anomaly in the study of epithelial cancers. The establishment of epithelial cell lines for the study of carcinogenesis has historically faced the phenomenon of the emergence of fibroblasts along with the loss of the proliferative potential of epithelial cells ¹¹¹. Even current attempts for the *ex vivo* evaluation of the chemosensitivity of tumors deals with the contamination of the samples by mesenchymal cells. Interestingly, the fibroblasts are more resistant to drugs than epithelial cells derived from the same tumor ¹¹². Despite the findings, at best, the fibroblasts remain relegated as secondary players in epithelial carcinogenesis.

If the hypothesis is correct, the low-grade epithelial tumors are benign tissues in their way of transformation. This implies that the real carcinoma begins once a certain threshold of restrictions is surpassed and the events

of transdifferentiation and the stabilization of the mesenchymal phenotype are favored. This stage would correspond with the high-grade carcinomas. These assumptions also explain the large difference in survival between the well-differentiated tumors versus the high-grade carcinomas. Therefore, is theorized that carcinomas are lethal once the mesenchymal phenotype is reached and stabilized. The previous stages are part of the necessary process for the *in vivo* reprogramming. The proposal that the origin of carcinoma lies in the mesenchymal cells that arise from senescence in epithelial hyperplasia can solve another recognized paradox. In the presence of TGF β , the normal epithelial cells arrest their cell cycle. Thus, this cytokine was considered a potential effective anticancer agent in the management of carcinomas. The studies showed that it restrains the growth in early-stage tumors, however, an opposite effect resulted in advanced carcinomas. It was logical to justify the apparent dual activity to the loss of the regulation in the TGF β pathway during carcinoma progression, in which tumor cells shifted the signal into proliferation and invasion ¹¹³. According to the hypothesis is expected that the TGF β presents the ability to foster tumor progression in advanced stages if carcinomas are mesenchymal driven diseases. Observations from the immortalization of normal epithelial cells indicate that the ability to sustain growth in presence of TGF β develops along with the adoption of a fibroblast-like morphology ¹⁰². This is also consistent with the carcinoma cell lines, in which the ability to induce tumorigenesis is reserved for the cells with fibroblastic morphology ¹¹⁴. It is also not surprising that the proliferation of mesenchymal stem cells can be stimulated by TGF β signaling ¹¹⁵. The unavoidable progression of carcinomas towards independence from ligands explains the temporal responses observed using hormonal agents or targeted therapy ¹¹⁶. The process is naturally understandable if the mesenchymal undifferentiated cells are considered the phenotype responsible for carcinomas. Furthermore, is unnecessary to invoke complicated mutational molecular mechanisms to understand the process of progression as a whole, since the induction of cellular transitions allows a discrete change in the cellular phenotype along with the shift into autocrine signals, the lack of anoikis, and the dedifferentiation of the epithelium. Additionally, the mesenchymal cells that arise from senescence in epithelial hyperplasia emerge with stemness and the ability to induce cancer, but more importantly, they explain the cellular plasticity at play in the advanced stages of carcinoma. Based on the hypothesis, is next provided an attempt to understand the underlying order in the middle of complexity at play during the histological progression of carcinomas.

4. The origin of carcinoma

4.1. More than mesenchymal

The process of EMT was temporally viewed as a mechanism reactivated by tumor cells to acquire an invasive phenotype. However, an increased number of experiments found that EMT also explains the cancer stem cell phenotype ¹¹⁷, the resistance to chemotherapy ^{118, 119}, the immune system evasion, and the ability to induce tumors and their heterogeneity ¹²⁰. Consistent with the hypothesis, mesenchymal cells that emerge from cellular transitions are not simple fibroblasts, the empirical evidence confirms that EMT generates cells with the expression of the set of pluripotent genes, such as, SRY-Box Transcription Factor 2 (SOX2), the homeobox pluripotency transcription factor (Nanog), the octamer-binding transcription factor 4 (Oct4), the RNA-binding protein that regulates mRNA translation in embryonic stem cells (Lin28B), and the neurogenic locus notch homolog protein 1 (Notch1) ^{17, 121}. Despite being a small proportion of the tumor, the cancer stem cells are considered the main culprits of tumor relapse after cancer therapy ¹²². Their functional characterization to seed new tumors have been reviewed by the analysis of expression of cell surface markers, the formation of mammospheres, and using the xenotransplantation limiting dilution assay ^{123, 124}. The induction of EMT by inflammatory cytokines also generates cancer stem cells, and carcinomas show a correlation among the biomarkers of the process of inflammation, EMT, and stemness. The increased levels of proinflammatory cytokines *in vivo* has been linked with the induction of EMT and the accumulation of cancer stem cells in tumors ¹²⁵. Notwithstanding extensive empirical evidence indicating that the EMT might be the source of most aspects relevant for cancer, its contribution to *in vivo* carcinogenesis remains at debate

¹²⁶. Since high-grade carcinomas are not a homogenous mass of mesenchymal stem cells and the metastasis manifest an epithelial phenotype, to support the hypothesis it is necessary to explain the generation of heterogeneity and metastasis. In the case of tumor heterogeneity is almost self-explanatory since the process of EMT originates fibroblasts, cancer stem cells, vascular endothelial cells, and pericytes ¹⁰⁹ (reviewed in section 6). On the other hand, the change in microenvironment enables the MET of tumor cells and offers a compelling origin for the epithelial phenotype of metastasis. In the hormone-independent breast cancer cells MDA-MB-231, which present *in vitro* a mesenchymal phenotype, the metastatic lesions that generate in mice express the protein E-cadherin and display an epithelial phenotype ^{127, 128}. Furthermore, the process of MET was triggered by the interaction of cells with the parenchyma of the lungs and bones. At the molecular level, the reversion of EMT involves the negative regulation of the mesenchymal transcription factors product of the downstream signals of the microenvironment while the epithelial proteins are activated ^{129, 130}. As is now recognized, the induction of MET *in vitro* can reprogram fibroblasts into induced pluripotent stem cells (iPSCs) that showed the expression of the transcription factors Oct4, the zinc finger-containing transcription factor Krüppel-like factor 4 (KLF4) along with the repression of the Snail ¹³¹.

In general, the induction of epigenetic reprogramming *in vivo* as a result of the histological changes in the hyperplasia suggests a plausible sequence of events for the reactivation of developmental programs that may account for most observations during carcinogenesis. According to the hypothesis, at least two cellular transitions are required for the generation of cancerous cells, however, the tumoral progression towards high-grade carcinoma would need conditions in the tissue that foster the constant generation and maintenance of the dedifferentiated cell states.

4.2. The Tissue Matters

The accumulation of senescent cells over the years compromises the renewal, structure, and function of the tissues. Eventually, the burden of senescent cells in the hyperplasia would reach a threshold of inflammation in which the cellular transitions towards the mesenchymal stem phenotype are attained. However, the maintenance of the cancerous behavior and the increased rate of cellular transitions would need a permissive microenvironment for carcinogenesis. This stage, perhaps, requires an extra input of proinflammatory cytokines along with the irreversible loss of the extracellular matrix and cell-cell interactions. Hence, the effects of senescence and inflammation in tissue architecture may explain the increased susceptibility to carcinogenesis of the aged epithelial tissues.

The contribution of senescent cells to structural disruption include the secretion of epithelial growth factors and proinflammatory cytokines that enhance the mitosis of epithelial cells and its eventual entry to senescence ^{132,133}. The monocyte chemoattractant protein 1 (MCP1) ¹³⁴ is also liberated by senescent cells and upregulates the migration and infiltration of monocytes, lymphocytes, and NK cells ¹³⁵. According to the hypothesis, is possible that macrophage activation plays a major role in the progression of hyperplasia since its transformation is consistently associated with their infiltration ¹³⁶. Macrophages are essential in normal development and repair via tissue remodeling. Unlike the common view that tumor-associated macrophages are a special type of cells reprogramed to sustain inflammation and tumor growth ¹³⁷, here is implied that the positive feedback loop that emerges between senescent cells and the activated macrophages synergize for the pathological transformation of the tissue in part by their production of MMPs and the degradation of the structural constraints that normally impose a regulated cell behavior. The substrates of the MMPs include many proteins in the extracellular matrix, proteinase inhibitors, cell surface receptors, and cell-cell adhesion molecules ¹³⁸. Both cell types express several MMPs that allow the cell detachment from the extracellular matrix, the basal membrane, and cells ¹³⁴. In this regard, clear evidence of the capacity of mechanical forces to induce pluripotent stem cells has been presented ¹³⁹. The cell phenotype is coupled to cellular morphology and stromal interactions through the signaling pathways that sense the mechanical stress of the cytoskeleton by the focal adhesion kinase (FAK) that activates the transcription factor Yes-associated protein (YAP) and the transcriptional co-activator with PDZ-binding motif (TAZ). These in turn are translocated to the nucleus and

promote the transcription of genes associated with cell growth, resistance to apoptosis, and carcinogenesis. Furthermore, the activation of the transcription factors YAP and TAZ is sufficient to induce cancer stem cells¹⁴⁰. Currently, it is considered that tumors also reprogram normal fibroblast into cancer-associated fibroblast since the only detected differences in them are the epigenetic pattern of methylation¹⁴¹. The shift is associated with a constant activation of NF- κ B and the secretion of TGF β , IL6, MCP1, the vascular endothelial growth factor (VEGF), and MMPs. These secretions act autocrine, paracrine, and modify the organization and the physical properties of the tissue microenvironment to promote fibroblast proliferation, the infiltration and activation of macrophages and inflammation, the induction of EMT, metastasis, angiogenesis, and the enrichment of cancer stem cells in the tumor microenvironment¹⁴². On contrary, according to the hypothesis, the cancer-associated fibroblasts correspond mostly with cells that arise from EMT, either from inflammation or as the result of the stromal disruption, and would explain the gradual loss of senescent cells and the increased stromal content during the progression to high-grade carcinoma. This stage implies the acquisition of tumor autonomy and perhaps marks the irreversible arrow of carcinoma progression towards dedifferentiation.

Overall, it is suggested that the first step in the origin of carcinomas involves the combination of an increased number of senescent cells with the secretory phenotype, chronic inflammation, and mechanical disruption in the hyperplasia. Then, its progression is allowed by the sustained levels of inflammation, the irreversible loss of normal tissue architecture, and the amplification of the mesenchymal phenotype. The higher levels of proinflammatory cytokines combined with relatively few possibilities of interactions with epithelial cells, and the normal stroma that otherwise would prevent the cancerous phenotype results in the inexorable progression to high-grade invasive carcinoma. In these tumoral conditions, the mesenchymal and the undifferentiated cell phenotypes are preferentially sustained. However, the interaction of these cells with microenvironment clues from the stroma, cells, parenchyma, and the oscillation of the endocrine milieu, such as the concentration of oxygen, nutrients, and growth factors or the reduction in the levels of inflammation explain the tumor heterogeneity and the formation of metastasis. On the other hand, it is likely that some dedifferentiated cells that emerge within the tumor migrate and may differentiate into functional cells in healthy tissues, and most importantly; the possibility that the underlying reason for the targeting of senescent cells by the immune system, is the attempt to regenerate the tissue. In this sense, the origin of carcinoma is the pathological consequence of a normal endogenous process of tissue regeneration.

5. Inflammation and cellular plasticity

5.1. Epithelial regeneration by cellular transitions

The notion of cancer as the result of overhealing is by no means new to pathology. As a result of the hypothesis, a more encompassing view of epithelial carcinogenesis emerges and suggests an endogenous mechanism by which the immune system undertakes the maintenance and rejuvenation of tissues by the epigenetic reprogramming of damaged cells.

Inflammation has been linked to the pathophysiology of carcinomas and with almost every hallmark of the cancer cell. Tumor tissues exhibit high levels of expression of cytokines compared to normal samples, and their presence is also positively correlated with advanced tumor grade. Elevated serum levels of inflammation are associated with adverse prognosis and an increased number of metastases. The subsequent discovery that cytokines can induce EMT and pluripotency in cancer cells renewed the interest in the relationship between inflammation and cancer from a different perspective¹⁴³. However, it is important to notice that normal epithelial cells exposed to interleukins experience the same effects^{17, 144}. This implies an endogenous mechanism by which inflammation induces cellular plasticity. In such a scheme, the outcome of the epigenetic reprogramming *in vivo* of senescent cells might result in a recovery of the proliferative potential along with normal epithelial phenotype. In the youth epithelia, the presence of stromal and cell interactions

ensures the normal differentiation of the reprogrammed cells and the outcome is the clearance of the secretions of senescent cells and the resolution of inflammation.

Circumstantial evidence is emerging regarding the role of senescence, EMT, and inflammation in the regeneration of tissues *in vivo* using epigenetic reprogramming¹⁴⁵. It turned out that the secretory phenotype is essential for tissue repair, fibrosis reduction, and wound resolution¹⁴⁶. Furthermore, the locus Ink4b/ARF/Ink4a and the production of IL6 were crucial for the induction of EMT and the expression of the transcription factors cellular Myc (c-Myc), Oct4, Sox2, Klf4 during cell reprogramming and wound regeneration *in vivo*¹⁴⁷.

The results can be interpreted as a mechanism of tissue renewal triggered by the senescent cells and inflammation to induce epigenetic plasticity for the recovery of injured cells. In this scenario, the senescent cells in the hyperplasia are cells with DNA damage that became inflamed, activate NF- κ B, and release proinflammatory cytokines and chemokines. The secretory phenotype attracts and activates the immune cells that generate higher levels of tissue disruption and production of bioactive mediators. These situations would promote the EMT in senescent cells since intrinsically are prone to reprogramming due to epigenetic derepression. This event would allow access to an endogenous cell state with the abilities of self-repair, then, the interactions of the dedifferentiated cell state with the normal tissue enable the proper instructions for the attainment of the cell fate. Hence, stemness is transient, and the inflammation is resolved in the absence of DDR. On the other hand, the combination of senescent cells with extended molecular damage and aberrant structural microenvironment would sustain the generation of mesenchymal cells and the properties of cancer stem cells. The lack of resolution of this attempt of healing would become the unavoidable histological progression towards the lethal high-grade carcinoma. Hence, the origin of sporadic carcinomas is assumed as the consequence of the endogenous process of healing in aged and inflamed tissues. The argument presented above is based on common observations in carcinomas although from a different insight. In the next section it is compared with the prevalent paradigm, alternative proposals, and then is provided a unified view of epithelial carcinogenesis.

5.2. Cellular plasticity unifies the models of carcinogenesis

There are several features inherent to cancer, such as the genetic and chromatin damage, the disruption of tissue during the formation of tumors and metastasis, the multistep nature of carcinogenesis, an association with aging and inflammation, and the remarkable similitude with the process of wound healing and embryo development¹⁴⁸. Accordingly, the models that have been proposed in the history of cancer research adopted those observations into their premises. Through the years the dominant paradigms evolve, but essentially the new findings were customized into their original assumptions. The following provides a brief description of each, its premises, and their interpretations of the evidence.

The somatic mutation theory posits that the accumulation of mutations in the genetic material of epithelial cells is favored as a function of time and the increased levels of inflammation. The effect of telomere attrition, chemical exposure, infections, or radiation would be additive in the accumulation of mutations and the chances of acquisition of malignant traits¹⁴⁹. However, most of the malignant features arguably conferred by oncogenes and tumor suppressors play essential roles in the normal biology of cells¹⁵, the random events of mutations and Darwinian selection hardly explain the transcriptional convergence of high-grade cancers¹⁶, and carcinogenesis does not require mutagenesis¹². Additionally, the reversion of the malignant behavior²¹ and the acquisition of the cancer stem phenotype through events of transdifferentiation¹⁷ challenges entirely the logic of this theory. The whole cancer phenomenon is attributed to a series of fortuitous mutagenic events in different cell phenotypes which then are associated with a fixed purpose in carcinogenesis. Hence, it demands the creation of at least the cancer version of the normal stem cells, macrophages, fibroblasts, or the pathological variants of senescence, EMT, and inflammation. As a result of the inconsistencies, this paradigm is moving towards epigenetic mechanisms to sustain most of its original premises.

The importance of tissue architecture in cancer development was neglected by the somatic mutation theory although adapted to some extent by considering the effects of tumoral cells under its microenvironment ¹⁵⁰. The theory of the field of tissue organization addresses this, being the stroma the primary target of carcinogens. The tumorigenesis of epithelium is explained by considering that tissue disruption releases the inherent proliferative and motile behavior of normal cells. Hence, provides an explanation for the induction of differentiation when tumors are exposed to the normal stroma, as well as the opposite effect of the acquisition of malignant behavior of normal cells in response to abnormal stroma ¹⁵¹. Although offers no integral explanation for the process of carcinogenesis, exposes the limitations of the somatic mutation theory, and offer some relevant premises, for example, that tissue integrity throughout aging diminishes, that mutations are unrelated to causality, that cancer is a developmental process, and the possibility of tumoral reversion.

On the other hand, the notion of cancer as a deregulated process of healing takes into account the association of chronic inflammation, fibrosis, and tissue disruption with carcinogenesis ¹⁵². Wound healing shares many features with tumorigenesis, such as the presence of activated immune cells, cell migration, angiogenesis, fibroblast, myofibroblast, and cell proliferation, the generation of stroma, the induction of EMT, and the production of mediators for tissue remodeling. Despite the suggestions that hyperplasia may transform by overhealing, and that tissue regeneration and carcinogenesis are linked at the molecular level, findings regarding this posture remained mostly descriptive.

The view of cancer as a developmental disease highlights that embryo and tumor development are parallel processes since both originate from a single cell that undergoes accelerated proliferation and migration using the same mechanisms. Since the nineteenth century, pathologists suggest that tumors are a deregulated process of development, and using modern molecular techniques was confirmed the reactivation of the mechanisms normally associated with embryo morphogenesis ^{16, 153, 154}. However, the identification of the EMT, MET in the generation of cancer stem cells and metastasis, and the embryonic transcriptional profile of high-grade carcinomas was mainly relegated as the result of the promoting effects of the tumor microenvironment over the mutated cancerous cells.

Regarding the position of cancer as an embryonic or primitive preexistent cell-state, the view of cancer as an atavistic state posits that in case of adverse conditions, cells reactivate an ancestral genetic program that was suppressed during evolution. This cellular state would correspond with functions associated with survival as a unicellular form. Hence, mutations may reactivate an endogenous behavior already primed for carcinogenesis ¹⁵⁵. Likewise, the proposal of cancer attractors suggests that cancer is the result of cellular states closely related to embryonic programs, but unable to differentiate. In this context, mutations or microenvironment perturbations would allow access to the cancerous phenotype ¹⁵⁶. Both perspectives agree on the undifferentiated nature of the cancer cell, and that carcinogens would promote the activation of an endogenous cellular state.

The main hypotheses of carcinogenesis have been integrated or overlapped in various degrees through the years. Invariably, most of them coincide that the event of genesis is associated with chromosome instability or genetic damage. The importance of mutations was greatly influenced by the study of the mutagenic effects of viruses or chemicals that induce cancer. Hence, cancers should probably originate from mutations in genes of DNA repair. These ideas were then incorporated in the two-hit model, which established the need for a second key mutation for tumor progression especially in genes that control cell proliferation ¹⁴⁹. That model prevails and is used to understand the multi-step nature of carcinogenesis. In other words, that most solid cancers are preceded by focal proliferative lesions, in the case of carcinomas the hyperplasia.

The different positions can be contextualized in a unified view of epithelial carcinogenesis. Telomere attrition due to replicative exhaustion justifies the presence of DNA damage in cells that originate the tumor. Instead of considering that the cancerous phenotype is created by mutations, selective silencing, or activation of genes, is proposed that this event generates unspecific transcriptional derepression that in the first instance lead to a discrete change in the cellular phenotype from which emerge the senescent cell. Chromosomal anomalies accumulate in senescent cells, which number increase in aged tissues and raise the level of immune

cell infiltration due to the secretory phenotype. Those events explain the long relationship of aging and inflammation with the susceptibility of carcinoma development. Moreover, imply the multistep process of carcinogenesis since the hyperplasia provides the conditions for the *first hit* necessary for the transformation, but still is insufficient for the invasive and lethal behavior. Then, the higher burden of senescent cells and the infiltration of immune cells contribute to the structural changes in the stroma and the loss of cell interactions that allow deregulated *in vivo* reprogramming that essentially drives progression. This stage would correspond in a sense, with the *second hit*, the disappearance of senescent biomarkers, and the rise of proteins associated with inflammation, EMT, and cancer stem cells. In this setting, the proposed series of cellular transitions and the progression towards dedifferentiated carcinoma partially relate with some of the premises of the theory of the field of tissue organization and with the view of cancer as a developmental disease that is the consequence of endogenous programs and undifferentiated phenotypes.

The increased transcriptional derepression of somatic cells in response to endogenous molecular damage or extracellular interactions results in the emergence of a mesenchymal stem-like phenotype that would drive the genesis and progression of the sporadic carcinomas. In the setting of the high-grade lesions or large-sized tumors, the disruption of the tissue and the high levels of inflammation are enough to stabilize the cancerous phenotype and explain the lack of differentiation and the autonomy that epitomizes the advanced carcinoma. The hypothesis of the origin of carcinomas presented in this paper might be considered a model since is rooted in observations and proposes mechanisms. In the context of this model, carcinomas can be collapsed for simplicity into a series of cellular transitions in which the original cellular phenotype, the magnitude, and type of perturbation govern the level of cellular plasticity (Fig. 3.).

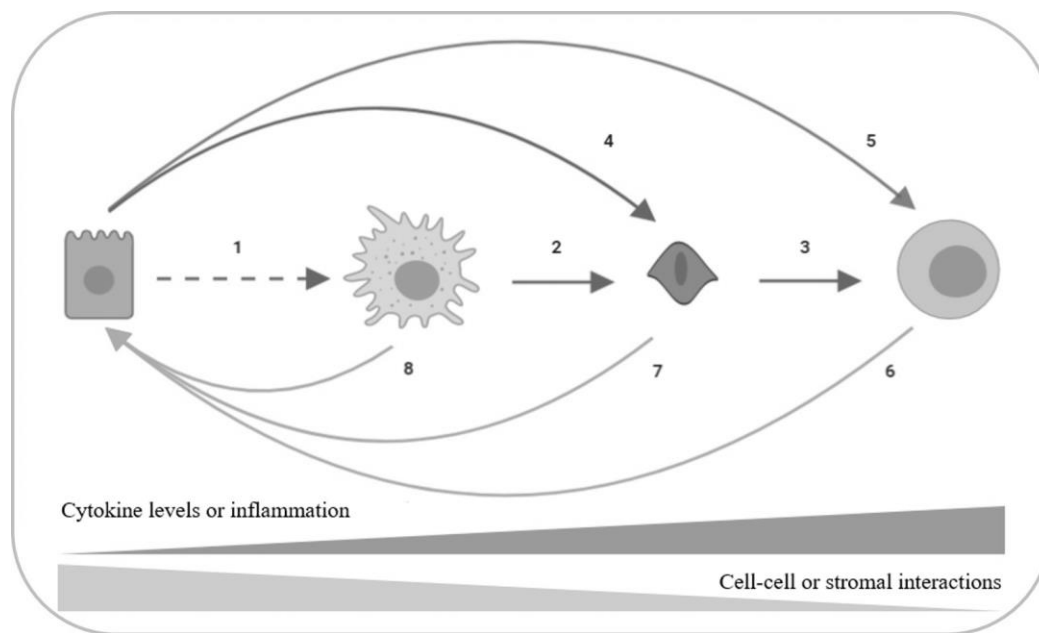


Fig. 3. Inflammation and cellular plasticity.

1) Studies *in vitro* have shown that cytokines induce mitosis of competent epithelial cells until they enter replicative cell senescence. 2) Cells can escape cell arrest and acquire fibroblast morphology using EMT. 3) Cells undergoing EMT can achieve the stem cell phenotype if they continue exposed to high concentrations of proinflammatory cytokines. 4) Exposure to high levels of inflammation can induce EMT in normal epithelial cells and result in the emergence of mesenchymal stem cells. 5) The epigenetic reprogramming of epithelial cells into induced stem cells is possible with inflammation. 6) Stromal interactions induce several grades of differentiation and explain tumor heterogeneity, 7) the formation of metastasis by induction of MET, 8) and the maintenance and healing of aged tissues. In the main text is provided empirical evidence of each cellular transition.

According to the hypothesis, the main driver in the discrete switch in phenotype associated with the cellular transitions underlying carcinogenesis is epigenetic regulation. In line with the premises of the theory, growing evidence points out that the transcription factor NF- κ B integrates the stimulus from proinflammatory cytokines and the structural change in tissues or the chromatin damage with the players of the epigenetic regulation.

5.3. NF- κ B links inflammation with cellular plasticity

Cancer cells are characterized by extensive epigenetic changes compared to their normal counterparts. Epigenetics broadly refers to mechanisms that mediate the differential access to the genetic material by the modification of the chromatin structure, namely, stable changes in gene expression that are independent of the nucleotide sequence. Transcription is regulated by the degree of compaction between histones and genes through the covalent modification of DNA or in the nucleosome and ATP-dependent chromatin remodeling. Hence, the epigenetic marks create several genetic profiles from the same genetic code that govern the singularity of each cell phenotype. The temporal regulation of the gene expression by the epigenetic process is central for the appropriated cell fate commitment and differentiation¹⁵⁷. The epigenetic control mediated by the enzymatic alteration of the structure of the chromatin is one of the most studied mechanisms in the network of transcriptional regulation. The activity of DNA methyltransferases (DNMTs) results in transcriptional repression due to chromatin compaction by the methylation of cytosines. The proteins that modify the histones include methyltransferases (HMTs) and acetyltransferases (HATs). Histone methylation occurs in lysine and arginine, whereas acetylation, on serine and threonine residues. The polycomb proteins also mediate gene silencing by the formation of the polycomb repressive complexes (PRC) PRC1 and PRC2 that change the configuration of the chromatin and prevent the transcription. In the PRC2 the histone lysine methyltransferase EZH2, acts together with the zinc finger protein SUZ12 (suppressor of zeste 12 homologue) to catalyze the trimethylation on K27 histone H3 (H3K27me3), which is then recognized by PRC1, with members such as the proto-oncogene Polycomb Ring Finger (Bmi1) protein that maintains chromatin silencing. Is considered that the more stable form of epigenetic repression and to maintain the cell identity and differentiation is the silencing of genes by H3K27me3 and PRCs. However, the epigenetic marks can be reversed by the activities of DNA demethylases (DNMTs), such as the Ten Eleven Translocation enzyme (TET), the Lysine-Specific Demethylase 1 (LSD1), and the Jumonji C-terminal domain (JmjC), both histone lysine demethylases (HDMs), and the histone deacetylases (HDACs), such as HDAC1 and HDAC2, and Trithorax proteins (TRXG) that counteract the repression by PRCs¹⁵⁷.

The molecular mechanisms necessary to explain the events of transdifferentiation begin to be understood. Interestingly, increasing reports suggest that NF- κ B activation can erase cell identity through the induction of enzymes that reshape the chromatin and enables the epigenetic reprogramming that manifests as cellular plasticity that underlies carcinogenesis according to this manuscript.

Despite the activities of NF- κ B are usually viewed as a process exclusive to the immune system or with tumor progression, it is ubiquitously expressed, and its inhibition is lethal to any cell type. NF- κ B suppresses the pathways of cell death in normal and cancer cells and is essential in the regulation of normal epithelial homeostasis¹⁵⁸. Since the NF- κ B pathway is induced in response to changes in the environment, including cytokines, radiation, oxidative stress, hormones, or growth factors, and by intracellular damage is comprehensible its hyperactivation in cancer¹⁵⁹. The NF- κ B transcription factor family has five members: the transcription factor p65 subunit (RelA), the cellular homolog of v-Rel (c-Rel), the V-Rel Avian Reticuloendotheliosis Viral Oncogene Homolog B (Rel B), the DNA binding subunit of the NF- κ B (p50), and the NF- κ B p52 subunit (p52) which form various homo and heterodimers. In the resting state, NF- κ B dimers are in the cytoplasm in an inactive form, through their binding to inhibitory proteins known as inhibitors of κ B (I κ B α , I κ B β , and I κ B ϵ). The majority of stimulus that leads to NF- κ B activation converge on the I κ B kinase (IKK) complex, which is responsible for I κ B phosphorylation and allow the nuclear translocation of

NF- κ B. Its transcriptional activities increase the expression of more than 500 genes, among them, the chemokines, lymphokines, interferons, acute phase proteins, and their receptors, the enzymes cyclooxygenase 2 (COX-2), lipoxygenase 5 (LOX-5), and the nitric oxide synthase (NOS). The antiapoptotic proteins such as the B-cell lymphoma 2 (Bcl-2), the Bcl extra-large (Bcl-xL), the Bcl-2-like protein 11 (Bim), the cellular FLICE-inhibitory protein (c-FLIP), the X-linked inhibitor of apoptosis protein (XIAP), the membrane proteins ATP-binding cassette transporters, the P-glycoproteins, the growth factors FGF8, the hepatocyte growth factor (HGF), the bone morphogenetic protein 2 (BMP-2), BMP-4, the nerve growth factor (NGF), VEGF, the placenta growth factor (PIGF), the epidermal growth factor receptor (EGF) and the human epidermal growth factor receptor 2 (HER2). The transcription factors c-myc, the homeodomain transcription factors (HOXs), the proto-oncogene AP-1 Transcription Factor Subunit (JunB), p53, the E2F transcription factor 3 (E2F3a), Twist, the E74 like ETS transcription factor 3 (Elf3), and the chromatin modifiers Bmi1 and Jmjd3. Traditionally, the mechanism that underlies the regulation of distinct sets of genes by NF- κ B is attributed to its different dimers and the association with several families of transcription factors specific to each cell phenotype ¹⁶⁰.

The activation of the NF- κ B signaling pathway in carcinoma cell lines is linked with demethylation that results in the upregulation of genes normally expressed in pluripotent embryonic stem cells ¹⁶¹. Furthermore, the epigenetic reprogramming with the cytokines TGF β , TNF α , IL1, and IL6 increases the expression of the markers CD44, CD133, Nanog, Bmi1, Oct4, and vimentin ^{162, 163}. The molecular regulatory networks that coordinate the events of transdifferentiation such as the EMT and the generation of cancer stem cells by inflammatory cytokines involve the activation of Snail, Twist, Zeb, and epigenetic regulators by NF- κ B ¹⁶⁴. For example, the induction of Jmjd3 depends on the direct binding of NF- κ B to a cluster of three kB sites in its promoter. Then, Jmjd3 promotes site-specific H3K27me3 histone demethylation leading to transcriptional derepression and cellular plasticity ¹⁶⁵. The process of EMT involves the repression mediated by the PRCs of epithelial genes through the initial recruitment of Snail, which binds to the E-box elements of the promoters. Snail recruits PRC2 and interacts with EZH2 and SUZ12 to catalyze the H3K27me3 and silencing of gene transcription by PRC1. Snail also mediates the recruitment of LSD1 to target genes that can trigger EMT and cancer progression, in conjunction with additional epigenetic modifications. The formation of LSD1-Snail complexes on gene promoters catalyzes the removal of methyl groups from the H3K4me3 activation mark leading to the loss of transcriptional activation of epithelial genes. LSD1 is highly expressed in several cancer types and correlates with poor survival ¹⁶⁴. During EMT, Twist directly activates Bmi1 expression and confer the properties of cancer stem cells. The axis of NF- κ B, Twist, and Bmi1 is essential for the repression of the epithelial phenotype, the Ink4b/ARF/Ink4a locus, and to sustain the mesenchymal stem cell transcriptional signature ¹⁶⁶. Snail and Twist can repress gene activity through deacetylation of gene promoters by recruiting HDAC1 and HDAC2, which catalyze the removal of acetyl groups from lysine 9 and lysine 14 residues of histone H3. Snail also promote the proteasomal degradation of p53 by HDAC1 deacetylation and recruit the HMTs such as the euchromatic histone-lysine n-methyltransferase 2 (EHMT2) and the suppressor of variegation 3-9 homolog 1 (SUV39H1), which act cooperatively to catalyze the trimethylation of H3K9 that is required for the recruitment of DNMTs. In turn, leads to the methylation of cytosine and guanine linked by phosphate in a repeated sequence (CpG islands) in gene promoters that block the transcriptional activity by the histone lysine methyltransferases ¹⁶⁴. This mechanism was also found during the EMT induced by the TGF β signaling to repress the epithelial genes. In mammary epithelial cells, TGF β also showed the induction of Jmjd3, the removal of H3K27me3 marks, and the reactivation of the mesenchymal genes ¹⁶⁷. The activation of NF- κ B by IL6 triggers an autocrine loop sufficient for the epigenetic switch from immortalized breast cells to cancer stem cells ¹⁶⁸. IL6 can change the pattern of DNA methylation in cancer cells by inducing the expression of DNMTs. The IL6 pathway is required for the CpG island methylation in promoters to repress tumor suppressors such as p53, and the epigenetic reprogramming to drive cancer cells towards the stem phenotype ¹⁶⁴.

The mechanical disruption of tissues and the disorganization of cells also activate inflammation by coupling the signals of the integrin and Rac kinases with the NF- κ B pathway through the activation of the IKK complex¹⁶⁹. Further, the comparison of the breast cancer cells HMT-3522 that grow in clusters versus a variant of disorganized cells show the differential overexpression of 180 genes that share binding sites for NF- κ B. Additionally, the suppression of the transcriptional activities of NF- κ B restores the formation of clusters and the suppression of motility. The culture of HMT-3522 for 4 days in contact with an artificial ECM and the presence of an inhibitor of IKK results in the reversion of the malignant phenotype and the formation of structures with polarity and integrin expression¹⁷⁰. Related findings showed that breast cancer cells depend on the sets of genes activated by NF- κ B to sustain the basal phenotype and the invasiveness¹⁷¹. Hence NF- κ B governs the malignant cell phenotype but is a reversible process in the presence of normal stromal signals and downregulation of inflammation.

The induction of inflammation also comes from intracellular inputs. For example, following irradiation or chemotherapeutic drugs that induce DNA breaks, the ATM kinase is activated and phosphorylates H2AX, leading to the recruitment of several other proteins involved in the DDR. The activation of ATM phosphorylates the NF- κ B essential modulator (NEMO), which in turn, induces NF- κ B activity¹⁷¹. Furthermore, the hormone-independent phenotype of breast cancer cells requires the activation of ATM in response to H2AX foci in the chromatin. The potential protumorigenic consequence of a constantly activated DDR has been observed in the mammary gland, in which the induction of the cytokine IL6 activates NF- κ B and ignite a positive inflammatory signaling loop, that perpetuate the proliferative signaling and the acquisition of mesenchymal stem traits¹⁷¹.

Overall, mutations appear inadequate to explain the complex behavior and cell heterogeneity observed during epithelial carcinogenesis. Some attempts to justify the acquisition of elaborated instructions for malignancy propose as the source the chromosome abnormalities such as breakages, fusions, and aneuploidy¹⁷². Aneuploidy can be induced by local microenvironmental changes, confers increased survival, and is associated with inflammation and metastasis¹⁷³. Seems logical that large genomic rearrangements are more effective in inducing adaptation than single-gene mutations. Among the multiple factors that have been proposed to induce chromosomal instability and aneuploidy are telomere dysfunction, stress, and DNA damage that also increased during the histological progression of solid tumors. However, increasing evidence support that the activity of the chromatin modifiers underlies chromosomal instability and aneuploidy since neither aneuploidy nor mutations are exclusive of cancer cells. Thousands of mutations occur in differentiated cells of healthy individuals and appear to be essential in the physiology of normal cells. Regarding aneuploidy, although is associated with cellular senescence and telomere attrition, it also temporarily increases in response to a normal regenerative stimulus. For example, the number of aneuploidies in hepatocytes rise in function of proliferation to restore the liver mass *in vivo* but is reestablished to normal¹⁷⁴⁻¹⁷⁶.

Here, is proposed that the prominent presence of chromosomal aberrations in cancer is the result of chromatin rearrangements that lead to large-scale genetic changes that modify the expression of many genes at once and allow events of transdifferentiation according to environmental and intracellular inputs. The secretory phenotype of the senescence cells is ruled by NF- κ B activation in response to telomere attrition since is sensed as DDR. In turn, intracellular inflammation in senescent cells within the hyperplasia loss transcriptional repression that makes them prone to epigenetic reprogramming. At the level of tissue, the change in morphology of senescent cells, the immune infiltration, and the combined secretions of cytokines and MMPs disrupt the interactions with the extracellular matrix and among cells that otherwise would restrict the cellular plasticity. The gradual overthrow of the constraints at both levels result in the hyperactivation of the NF- κ B pathway and increased epigenetic plasticity that fuels the progression to high-grade carcinoma in which the mesenchymal stem states are self-sustained, amplified, and intrinsically motile. The selective

evolutionary advantage of linking the signal of adversity with transcriptional derepression is clear since cellular dedifferentiation would provide the necessary mechanisms for self-renewal and survival. However, in aged tissues, the chances for carcinogenesis clearly overthrow the process of healing. Hence, the irreversible nature of carcinoma progression is towards dedifferentiation and metastasis. In line with the premises adopted in this paper, the endogenous genetic damage and the aberrant tumor microenvironment lead to transcriptional derepression mediated by cellular inflammation. NF- κ B recruits and interacts with epigenetic regulators creating feedback molecular loops that regulate cellular plasticity and link inflammation with epigenetic reprogramming. The coupling of the extracellular and intracellular perturbations to epigenetic reprogramming by the degree of activity of NF- κ B and the magnitude of plasticity results in the dynamic and reversible nature of cellular transitions that have been challenging the attempt to conceptualize a fixed purpose for each of the processes or phenotypes involved in carcinogenesis. Furthermore, explains the elusive nature of EMT, MET, and the cancer stem cells during the histological progression of the carcinomas *in vivo*.

6. The enigma of myofibroblasts

6.1. The emergence of myofibroblast

The origin and the mechanisms that regulate the emergence, clearance, or persistence of myofibroblasts have been elusive. Myofibroblasts exert traction forces in the extracellular matrix and synthesize its components during connective tissue remodeling and epithelialization in wound healing and disappear afterward. However, in organ fibrosis, these cells are persistent and predict its progression to carcinoma. The proliferation of myofibroblasts is also present in tumor desmoplasia and is associated with poor prognosis¹⁷⁷.

The hypothesis predicts that cells in transit of the second cellular transition *in vivo* would exhibit a temporal hybrid phenotype between senescent and the mesenchymal cells. Interestingly, the stromal cells that correspond with this profile are the myofibroblasts that display the markers of replicative senescence and mesenchymal stem cells. Consistently with the proposal presented in this manuscript, myofibroblasts are not only involved in fibrosis and cancer but also well known for their transient appearance in the process of tissue homeostasis and wound healing. According to the hypothesis, the phenomenon of myofibroblasts is related to aging and inflammation of the parenchyma, in which mostly the resident epithelial cells undergo senescence and initiate the process of healing. However, in conditions of severe tissue disruption, inflammation, or catastrophic chromosomal damage the events of dedifferentiation are persistent. In such conditions, the myofibroblasts are robustly produced from several cell types and novel discoveries in the field of fibrosis unveil its intrinsic plasticity and its protumorigenic potential.

Cells that have been involved in the proliferation of the stroma are the resident fibroblastic and mesenchymal stem cells along with cells from the bone marrow and perivascularity. Using more recent technology, epithelial cells showed to account for most of the fibroblastic cells during organ fibrosis and in the desmoplasia associated with carcinoma¹⁷⁸. Hence, the process of EMT became the most studied cellular transition in cancer biology and fibrotic diseases associated with inflammation. However, the cellular plasticity endowed by inflammation not only underlies the process of transdifferentiation of epithelial cells into fibroblasts but also in endothelial cells, adipocytes, and pericytes. These cells can transdifferentiate by EMT in cells that can behave as fibroblasts, myofibroblasts, or mesenchymal stem cells. In the case of epithelial cells, the cuboidal shape along with the expression of E-cadherin and zonula occludens-1 (ZO-1) is lost and turn into a spindle-shaped cell that expresses the fibroblast-specific protein (FSP1) or the α -SMA in the case of the myofibroblast¹⁷⁸. Interestingly, the fibroblast detected in preneoplastic lesions and chronic inflammatory conditions also express the markers of senescence¹⁷⁹. These cells are also present in the injured tissues limiting the accumulation of fibrosis and facilitated their resolution and healing. According to the expression of α -SMA these cells correspond with myofibroblasts, that exhibit cellular plasticity and multipotency¹⁸⁰. In line with the continuum of cellular transitions proposed in this paper, those events

correspond with the transition of senescent cells by EMT into mesenchymal stem cells with self-renewal and multipotency, hence, that intrinsically harbors properties for healing or carcinogenesis.

The endothelial cells, multipotent monocytes, fibrocytes, pericytes, adipocytes, and local fibroblasts, also dedifferentiate into myofibroblasts in response to external or internal stimuli^{177, 181}. Increasing studies show that the adoption of the fibroblastic program confers the cells with the intrinsic potential to be activated for differentiation into subtypes of fibroblast-like cells and several non-mesenchymal lineages. Hence, the current view of fibroblasts is as not terminally differentiated cell types, that exhibit heterogeneity in response to differences in the endocrine milieu, the neighboring cell phenotypes, mechanical forces, and its position in the tissue. The ability to sense the changes in the ECM, the autocrine and paracrine signals influences the epigenetic regulation that translates into transcriptional changes and may explain the plasticity of fibroblasts^{182, 183}.

The induction of EMT and the maintenance of the fibroblastic phenotype in adult tissues is associated with the stimulation of soluble growth factors, cytokines, and the influence of the extracellular matrix. In response to those events, the resident epithelial cells and fibroblasts can dedifferentiate into myofibroblasts¹⁸⁴. During fibrosis, the ligands of the Wnt, Notch, and Sonic hedgehog pathways, such as the TGF β , BMP, EGF, HGF, and FGF are commonly involved in the regulation of myofibroblast differentiation. The onset of fibrosis has been associated with changes in the mechanical forces of the tissue that activate the fibroblasts and the production of TGF β that in turn induces the proliferation of myofibroblasts and the accumulation of collagen¹⁸⁵. In carcinomas, the differentiation of fibroblast to myofibroblast and its proliferation is also regulated by TGF β . Regardless of their origin, myofibroblasts display a malignant phenotype resistant to apoptosis¹⁸⁶. In other chronic inflammatory diseases such as rheumatoid arthritis, the fibroblasts that proliferate in the affected joints also resemble immature malignant cells. They behave differently from normal fibroblasts, shown invasive potential and properties of mesenchymal stem cells. Furthermore, it has been suggested that resident stromal cells undergo dedifferentiation *in vivo* by the conditions of hypoxia and proinflammatory cytokines in the affected joints¹⁸⁷.

6.2. The multipotency of fibroblasts

The fibroblasts represent the heterogeneous mesenchymal cells which are extensively found in conjunction with almost every cell type as the primary system of interstitial support. Classically, is considered that the stromal connective tissue found in all organs is derived from mesothelial tissue and that the function of the fibroblasts is mainly to synthesize the ECM including the collagen, proteoglycans, fibronectin, laminins, glycosaminoglycans, and in the formation of scar tissue. However, current evidence supports their active role in restoring the function of tissues by transdifferentiation into several cell types¹⁸³.

Vimentin is the major structural component of the intermediate filaments used for fibroblast identification, which functions include contractility, migration, and proliferation. Other than vimentin and morphology, the biomarkers that precisely define the fibroblasts remain to be fully understood. The set of transcription factors that seem essential in the maintenance of the fibroblastic phenotype are the Paired related homeobox 1 (Prrx 1), the paired-related homeobox protein SHOT (Shox2), the AP-1 Transcription Factor Subunit (c-fos), Slug, and Twist1¹⁸⁸. However, during fibrogenesis, the mesenchymal cells acquire traits of embryonic cells and become highly proliferative¹⁸⁹. Furthermore, the origin of myofibroblasts is also tightly linked to upregulation of genes expressed during early embryonic development¹⁹⁰. In this regard, the consensus is that mesenchymal stem cells and fibroblasts can be distinguished by the differential expression of genes associated with developmental programs and the stromal functions. Mesenchymal stem cells show ubiquitous activation of the germline programs, with the activity of transcription factors of ectoderm, endoderm, and mesoderm. The transcription factors that differentiate them from normal fibroblasts are the expression of the ETS variant transcription factor 1 (ETV1), the ETV5, the forkhead box protein P1 (FOXP1), the GATA-

binding factor 6 (GATA6), the high mobility group AT-Hook 2 (HMGA2), KLF12, the PR-domain zinc finger protein 16 (PRDM16), the single-minded family BHLH transcription factor 2 (SIM2) and SOX11¹⁹¹. Moreover, the mesenchymal stem cells also possess higher grades of hypomethylation¹⁸⁰ and increased expression of the pluripotent marker Oct4 compared to fibroblast¹⁹². Therefore, seems logical to employ fibroblasts to obtain mesenchymal stem cells. A task that has been successfully accomplished *in vitro* with the transcription factors SOX2, OCT4, KLF4, and c-MYC¹⁹³, or using a mixture of chemical inhibitors and growth factors¹⁹². However, fibroblasts are highly plastic by endogenous mechanisms. Their multipotency was first described in studies that show its chondrogenic differentiation in response to mechanical stimulation. Consistently, fibroblasts show transcriptional diversity and heterogeneity relative to the mechanical forces that experience due to structural changes, position, and the endocrine milieu in different tissues¹⁸². Furthermore, they share features that were considered exclusive of the mesenchymal stem cells. For example, they express the surface markers CD90, CD105, CD166, and upon appropriate conditions differentiate into adipocytes, chondrocytes, osteocytes, hepatocytes, neurons, myocytes, or pancreocytes^{194,195}. Hence, both are administered as a form of cell therapy for stroke, heart failure, COPD, liver damage, osteogenesis imperfecta, Duchenne, Muscular Dystrophy, and cosmetical procedures¹⁸³. Due to their inherent immunomodulatory properties are also used in inflammatory and autoimmune diseases and to fasten recovery¹⁹⁶. Their injection into affected tissues is followed by differentiation to functional cells¹⁹⁷. However, several studies show that mesenchymal stem cells also stimulate tumor development¹⁹⁸ and form teratomas *in vivo*¹⁹³, raising concerns about the safety of this type of therapy.

Fibroblasts are also the most used cells to obtain iPSCs. In turn, these cells can be cultured to produce induced mesenchymal stem cells (iMSC) using MSC medium, inhibitors of the kappaB kinase epsilon, the TGF- β /Activin type I receptor, BMP4, or growing the cells in precoated surfaces with gelatin, collagen, or synthetic polymers¹⁹⁹. Not surprisingly, an endogenous mechanism that induces the spontaneous transformation of fibroblasts is telomere attrition due to replicative senescence. As previously reviewed, the senescent program is essentially the same despite the original phenotype, and fibroblasts also remain in arrest while undergoing changes in morphology, gene expression, metabolism, and epigenetics. The early molecular events also involve the recruitment of proteins activated by DDR²⁰⁰ and the induction of NF- κ B²⁰¹. The careful examination of the effects of cell passage in fibroblasts founds a gradual decrease of methylation, differentiation, and the emergence of mesenchymal stem traits and tumor behavior. Fibroblasts undergoing cellular senescence arise with the molecular, structural, and contractile features of myofibroblasts. Additionally, the fibroblasts obtained from carcinomas are usually senescent and positive for the expression of the myofibroblast marker α -SMA^{179, 180}. Seems plausible that fibroblast dedifferentiation into mesenchymal stem cells with tumor behavior *in vivo* is driving by epigenetic rearrangements associated with cellular aging, inflammation, and structural cues. According to the hypothesis, in the setting of progression or the advanced stages of the disease, is expected that several cell types, including cells in the stromal compartment or derived from the bone marrow, can dedifferentiate to myofibroblasts with cancerous behavior by the same series of cellular transitions.

6.3. The EMT is linked to embryonic programs

The epithelial cells are the precursors of the endoderm and mesoderm using developmental pathways. During gastrulation, the epithelial cells acquire plasticity by EMT and adopt the fibroblastic phenotype and motility for normal embryo development. The activation of the mesenchymal transcription factors is mediated by the TGF β , Wnt, and Notch pathways and their proper functioning avoid death during gastrulation or defects in morphogenesis²⁰². The coupling of EMT with an embryonic transcriptome involves the machinery of epigenetic remodeling. The overexpression of Snail, Zeb, or Twist on normal, immortalized, or cancerous epithelial cells result in the activation of Bmi1, stemness, and tumor-initiating capacity. The process of EMT *in vivo* is followed by Bmi1 induction and the enrichment of stem markers in carcinomas. Bmi1 overexpression represses the effectors of cellular senescence and increases self-renewal and multipotency in

adult and embryonic stem cells. During EMT induced by inflammation, NF- κ B activates the mesenchymal transcriptional factors and the enzymes of epigenetic remodeling. The activation of Twist directly binds the Bmi1 promoter and confer cancer stem cell properties, represses the epithelial phenotype, the Ink4b/ARF/Ink4a locus, and preserves the mesenchymal stem phenotype²⁰³.

According to the hypothesis, cellular plasticity is the result of epigenetic derepression, in which the original cell type and the magnitude of the stimulus are the main factors that limit the degree of cellular reprogramming. It is likely that cellular plasticity is increasing as the physiological levels of cytokines rise and the restrictions imposed by the stroma diminishes. Hence, carcinogenesis *in vivo* is self-organized and the tangible evidence is the typical histological progression of carcinomas. Essentially, at the core of the advanced carcinomas is deregulated cellular plasticity due to inflammation and the result is the stemness. Tumors and metastasis are the natural consequence of the subsequent partial differentiation of cancer cells in response to environmental changes that restrain chromatin derepression. A great body of current literature supports a bigger role of physical forces, tissue homeostasis, cellular aging, and inflammation on epigenetics, cell behavior, and plasticity that may ultimately result in carcinogenesis.

Consistent with the model, studies have shown that maintenance of the fibroblastic shape, the malignancy, and stemness *in vitro* requires high levels of proinflammatory cytokines that upon decrease, induce the reversal into an epithelioid morphology¹⁸⁷. The mesenchymal stem cells also become heterogeneous due to spontaneous differentiation or maturation associated with the production of ECM^{204, 205}. The experimentally silencing of Sox2²⁰⁵, Twist1, or Slug decreases Bmi1 overexpression, abolishes stemness, the invasiveness, and enables the differentiation towards a luminal phenotype²⁰³. Hence, the connection of inflammation, transcriptional derepression, and stemness is likely the molecular basis of the cellular plasticity driving carcinogenesis.

Recent evidence shows that full malignancy is associated with cells that exhibit a hybrid phenotype. Their multipotency is enough to explain the tumor growth and cell heterogeneity *in vivo*²⁰⁶. The finding of the expression of markers from several lineages in the hybrid malignant cells is expected considering the current view of the molecular biology of stem cells. Furthermore, it implies a more comprehensive and unified view of cancer as the result of development.

7. Cancer and development

7.1. Transcriptional derepression and stemness

The study of stem cells has revealed that they do not express a specific set of genes or signaling pathways to sustain their multipotency. Instead, it is suggested that stemness is a transient trait, characterized by having many potentials but no specialization. The transcriptional hyperactivity is considered the main responsible for the plasticity of the stem cells and implies that it is a cell state produced as the result of repressing differentiation²⁰⁷. Furthermore, the analysis of embryonic stem cells showed the overexpression of genes related to chromatin-remodeling, transcription and that differentiation correlates with a reduction of transcriptional activity²⁰⁸. Consistently, the activity of the transcription factors Oct4, Nanog, Sox2, Klf4, and c-Myc collectively occupy 10% of the promoters in the human genome and repress genes implicated in differentiation. Moreover, the PcG, DNA methyltransferases, and stem transcription factors are associated with the upregulation of transcription in stem cells²⁰⁹. Another interesting feature is the structural coupling of the cytoskeleton with the nucleus of stem cells that allow the sense of mechanical signals from the cell microenvironment and translate them into transcriptional multipotency²¹⁰. In general, the emergence of stem cells and their maintenance is strongly influenced by environmental factors and the dynamics of cell growth, inducing heterogeneity in cell-cell interactions, cytokines, oxygen, growth factors, geometry, and mechanical

forces^{211, 212}. Some niches induce stemness *in vivo* by paracrine effects and mechanical interactions. Hence, the cell microenvironment plays a major role in stemness, and these cellular states cannot be defined in isolation. Conceptually, niches of stem cells can be viewed as domains in which a group of cells and their structural and endocrine interactions result in stemness. This is also consistent with their apparent elusive presence, its nature as a cell state that requires an induction, and its particular spatial location in tissues. Through the comparison of different stem cell systems, some traits suggest generic characteristics of the relationship between stem cells and their supporting niche *in vivo*. It is proposed that the stemness is inducing by a combination of proximity to the basal lamina and other cell types that activate the JAK-STAT, TGF β , and Wnt-signaling²¹³. The cultures of stem cells also indicate that stemness is a property induced by unspecific stressors. Among the conditions that induce and sustain stemness *in vitro* include hypoxia²¹⁴, oxidative stress²¹⁵, radiation²¹⁶, chemotherapy agents²¹⁷, DNA damage²¹⁸, ECM degradation with proteases, and the four pluripotency factors (Oct4, c-Myc, Klf4, and Sox2). Stem cells need to grow in serum-free culture media enriched with growth factors such as the BMPs, TGF β , TNF α , and under hypoxic conditions, otherwise, they start to differentiate²⁰⁴. In response to adverse non-lethal conditions, any cell can undergo stemness that enables access into survival mechanisms and process of renewal that are normally silenced during differentiation to optimally perform highly specific tasks associated with the different cell phenotypes that are necessary for multicellularity. Hence, seem that stemness is a transient robust cell state that can be accessed for self-preservation, but once the conditions are suitable for survival, cells can differentiate according to environmental cues. Furthermore, the normal biology of the stem cells is implicated in every aspect of the so-called hallmarks of cancer (Fig. 4.).

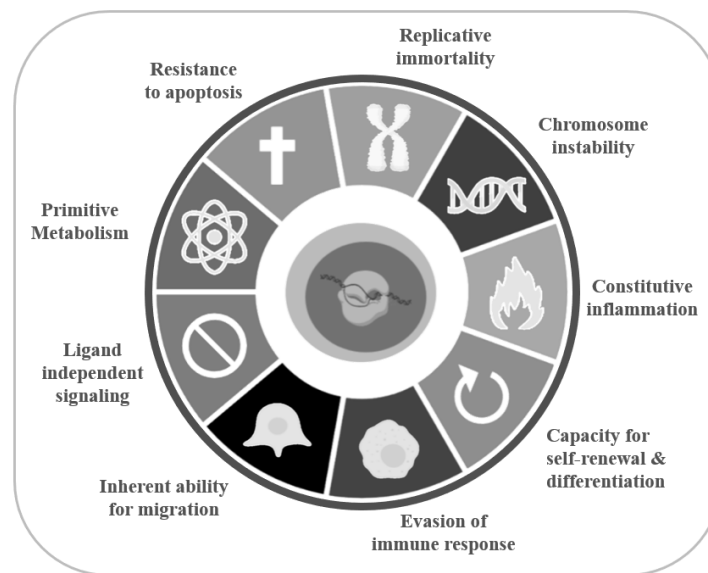


Fig. 4. Tumorigenic capabilities conferred by stemness.

Transcriptional derepression reconfigures several cellular processes largely associated with carcinogenesis. The metabolism of stem cells is characterized by the conversion of glucose to lactate despite the presence of enough oxygen to sustain the complete oxidation of glucose-derived carbons. These cells are heavily reliant on glucose and glutamine to proliferate^{219, 220}. Transcription factors that characterize stem cells downregulate p16 and p53 proteins preventing apoptosis^{221, 222}. Stem cells possess an increased mechanism of DNA repair, detoxifying enzymes, and overexpression of ABC transporters²²³. In stem cells, telomerase is activated and maintains telomere length, and confers cellular immortality²²⁴. Pluripotent stem cells showed chromosomal instability in long term cultures. Additionally, stem cells showed to acquire aneuploidy reminiscent of malignant cell transformation²²⁵. Stem cells constitutively activate NF- κ B (cellular inflammation) to sustain an undifferentiated cell phenotype¹⁶². Stem cells lack anoikis and have an inherent ability to migrate, which is as important as their capacity for self-renewal and to differentiate, enabling them to maintain tissue homeostasis and mediate repair and regeneration²²⁶. Additionally, stem cells acquire mechanisms for immune evasion²²⁷.

The comparison of cancer stem cells and embryonic stem cells reveals an absence of differences at transcriptional^{117, 228, 229}, and functional level¹¹⁷. Therefore, it has been suggested that the only difference between them is the microenvironment²³⁰. It is tempting to speculate that in parallel to normal stem niches, the epithelial tissues during carcinogenesis create aberrant compartments that foster transcriptional derepression and a deregulated access to stemness.

Cells that undergo senescence experience changes in chromatin, nucleus, and PcG proteins associated with transcriptional derepression. It can be argued that senescent cells are a step closer to stemness. Those features might underlie the reported closer epigenetic relationship between the senescent and cancer cells⁸⁶. According to the fundamental thesis in this paper, entry to senescence and tissue disruption during aging, in essence, make cells prone to dedifferentiation. Cellular aging reshapes the epigenetic landscape and links inflammation with regeneration and cancer. The possible outcomes are already codified in the genome and govern the entire course of carcinogenesis implying that the degeneration of tissues that underlies chronic diseases are part of the developmental process.

7.2. An unexpected, unified view of chronic diseases

Embryos originate from a cell that undergoes accelerated proliferation and migration through processes that parallels carcinogenesis. Long before the genomic era, the use of a simple microscope was enough to propose that cancer may be the product of an aberrant developmental process. In 1859, the pathologist Rudolf Virchow suggested that tumors originate on the same principles that operate during embryonic development. Later on, in the 1960s the ectopic implantation of normal embryos resulted in teratocarcinoma²³¹ and the injection of these cells into the blastocyst induced their differentiation in normal tissues¹². The cells extracted from these tumors were incorporated into functional tissues and expressed genes associated with differentiated phenotypes in healthy mice. In contrast, the cells that were kept *in vitro* were unable to express the sets of genes observed *in vivo*. Overall, those experiments exhibit an interesting facet of cancer; in which embryonic stem cells generate neoplasms in absence of mutagenesis and that carcinogenesis can be reversed by differentiation. Unfortunately, those results were regarded as rarities exclusives of teratomas and is likely that most modern molecular biologists are unaware. Furthermore, few researchers are even concerned about the inconsistencies in the current cancer field and rarely question their conceptual framework. Despite the historical delay, the late twentieth century witnessed the resurgence of evidence indicating that embryonic programs are involved in almost every aspect associated with cancer¹⁵⁴. Furthermore, an increasing number of studies in carcinomas correlate the infiltration of immune cells *in vivo* with the enrichment of embryonic stem cell markers and progression to higher histological grades²³². A remarkable finding was the transcriptional convergence into embryonic stem cell programs of high-grade tumors irrespective of the germinal cell layer or original tissue¹⁶. Additionally, stemness has been envisioned as the non-specific emergent product of the loss of the transcriptional repression²⁰⁷. Altogether, the evidence is consistent with the notion presented in this paper that carcinogenesis is a generic process in which somatic cells flow through cellular transitions towards dedifferentiation and stemness.

During early embryo development, when the embryonic totipotent cells reach the eight cell-stage, the first differentiated cell type that appears is the epithelial, and just a few divisions afterward arise the second cell type, the mesenchyme³³. During embryogenesis, senescence act as a transient form of plasticity in which cells eventually resume proliferation and contribute to the morphogenesis of tissues²³³. The process is broadly similar to the premises of the model, and suggests, that aging foster its reiteration by replicative senescence and chronic inflammation that may result in cancers. The robust emergence of the same basic cell phenotypes and the directionality of the observed cellular transitions suggests a genetic program that imposes the same functional and structural constraints to govern both, embryo development and epithelial carcinogenesis. Importantly, there is no reason to limit the argument to cancer since the emergence of most chronic diseases seems to follow the same order of cellular transitions.

Several tissues gradually undergo fibrosis with aging, including the skin, lung, liver, pancreas, and heart. Progressive fibrosis is a major cause of morbidity and mortality associated with repeated epithelial injuries and accumulation of myofibroblasts. The process of fibrosis is caused by the hyperproliferation of fibroblasts and the production of abnormal amounts of EMC that replace the normal structure of the parenchyma. The result is the functional impairment of organs and sometimes persistent fibrosis¹⁸³. The therapeutic options for organ fibrosis are handicapped by an incomplete understanding of the molecular mechanisms. Likewise, despite the current multitarget approaches to cope with cardiovascular diseases, diabetes *mellitus*, and cancers, they remain the principal causes of morbidity and mortality among adults in industrialized countries.

The model presented for the origin of carcinomas provides a mechanistic explanation of the underlying causes of fibrosis and steatosis accompanying the degeneration, the loss of function, and in some cases the transformation of tissues during aging from an evolutionary and molecular perspective. First, it is assumed that cellular senescence precedes the onset of most chronic diseases. Second, that inflammation and infiltration in the affected tissues accelerate their degeneration by increasing their susceptibility to cellular transitions. Finally, the cellular transition into mesenchymal phenotypes underlies fibrosis and steatosis. Being developmental biology the study of the mechanisms that govern the development of organisms from fertilization to senescence; epithelial carcinogenesis and chronic diseases seems to be part of the same processes that determine the loss of functions associated with aging. From an evolutionary perspective, the involution of the parenchyma makes sense since epithelial tissues are highly dependent on ligands produced by other specialized cells and are fine-tuned by structural interactions. In contrast, cells that undergo transdifferentiation into fibroblasts are more robust to adverse conditions. Thus, carcinogenesis could be viewed as an extreme consequence of the same conserved process that originates the rest of the chronic diseases, and the mesenchymal enrichment of tissues embraced as the logical consequence of the systemic conditions in aged patients.

The role of cells that undergo EMT or similar transitions during the fibrosis of vessels, hearth, liver, kidney, and pancreas is now increasingly recognized as an essential process for tissue degeneration in disease and aging^{234, 235}. The hypothesis implies that terminally differentiated cells can transdifferentiate into fibroblasts after senescence and explain the myofibroblasts expressing beta-galactosidase in the fibrosis of aged tissues. Thus, unifying the process of aging and chronic diseases under the same theoretical framework proposed for carcinogenesis. In essence, the hypothesis suggests that the involution of tissues is mediated by dedifferentiation and transdifferentiation *in vivo*. Furthermore, implies that the organized deterioration of tissues is precoded and the onset of chronic diseases and their manifestations are byproducts of the process. Consistent with this view, even when fibrosis is inevitable, the likelihood of tumorigenesis decreases by the downregulation of inflammation. Hence, the dynamics of aging and the associated diseases are ruled by development and its interplay with lifestyle. Overall, the cellular plasticity gained in aged tissues, their functional deterioration, and the eventual death is mainly the result of the unidirectional nature of DNA replication. Telomere length plays a major role in the possible number of cell divisions before the onset of a DDR response that induces cellular inflammation, transcriptional derepression, and ultimately cellular plasticity.

Regarding leukemias, its onset is preceding by the myelodysplastic syndrome that is characterized by cells with chromosome alterations and biomarkers of senescence²³⁶. Furthermore, the development of myeloproliferative disorders is associated with bone marrow fibrosis, and the leukemogens disrupt its stroma²³⁷. Upon evolution from chronic to acute leukemia, the chromosomal abnormalities increases, and the markers of cell dedifferentiation diminish²³⁸. In the blast crisis, which is the final stage of the disease, the cells become unresponsive to therapy and present stem cell gene signatures²³⁹. The overall process is then remarkably similar with the premises of this manuscript and suggest a principle. Although the details provided were oversimplified, and the molecular biology of each chronic disease on its own can be extremely complex; it emerges an underlying and valuable simplicity. If the theory presented in this paper is correct, some strategies of cancer prevention and therapy should be reassessed.

8. Reshaping cancer prevention and therapy

8.1. Cancer and lifestyle

The contribution of genetic factors in cancer development represents only 5% of cases, while the rest is attributed to environmental factors. Therefore, sporadic cancers are considered lifestyle diseases where most cases are associated with unhealthy habits or infections ²⁴⁰. Consequently, the policy for cancer prevention focuses on reducing the use of tobacco and alcohol, avoid obesity, the proper management of infections, and dietary advice ²⁴¹. Several investigations have addressed the potential mechanisms of lifestyle on cancer development. In this contribution, current knowledge was integrated to illustrate the global effect of lifestyle in epithelial carcinogenesis according to the hypothesis.

Diet is one of the most important factors for cancer development but also one of the most complex to understand. Even on a conceptual level, the term healthy diet is poorly defined. Nonetheless, several lines of evidence support that a diet based on fruits, vegetables, and whole grains is protective against cancer even in the presence of risk factors and reduces overall mortality among survivors. Importantly, the protective effect of a diet with an emphasis on plants extends to the rest of the chronic diseases ^{242, 243}. The concept of dietary pattern aid to define the type of diet associated with the protective effect in epidemiological studies. In the case of cancer, a healthy dietary pattern refers to a diet high in fruits, vegetables, poultry, fish, whole grains, and a low daily fat intake. In the opposite case, a diet with a high content of red meat, processed meats, refined sugars, sweet foods, and a high fat intake is considered unhealthy ²⁴⁴.

The effect of foods in blood biomarkers shows the integrated changes induced by dietary patterns in physiology and their potential impact on carcinogenesis. A healthy dietary pattern induces low levels of insulin, insulin-like growth factor (IGF-1), estrogen, testosterone, and proinflammatory cytokines such as TNF α , IL6, and C-reactive protein (CRP) ²⁴⁴⁻²⁴⁷. In turn, chronic exposure to low levels of these ligands results in low activation of the insulin receptor (IR), the mTOR protein (mammalian Target of Rapamycin), the estrogen receptor (ER), the androgen receptor (AR), the leptin receptor (Ob-R), and cytokine receptors, as well as their signaling pathways ²⁴⁸⁻²⁵⁰. Genetic, pharmacological, or dietary manipulation of these pathways increases longevity and delays the onset of all chronic diseases, including cancer ^{250, 251}. Healthy diets, being rich in plants, are also characterized by a low content of calories and fat, they are rich in fiber, therefore, they have a low glycemic index and are considered incomplete sources of amino acids ²⁵². Dietary interventions that involve those factors increase lifespan through mechanisms conserved in evolution. Examples include the caloric restriction, protein, or amino acid restriction (such as asparagine, glutamate, or methionine), or the ingestion of complex carbohydrates ²⁵³⁻²⁵⁵. The antiaging effects of these dietary strategies result in the negative modulation of the insulin / IGF-1 signaling pathway and that of mTOR, which are the most important regulators of lifespan in humans ²⁵⁶.

Healthy diets are also rich in bioactive phytochemicals such as terpenes and phenolic compounds capable of exerting direct effects on the extension of longevity by modulating insulin / IGF-1 signaling pathways, mTOR, activated protein kinase by adenine monophosphate (AMPK), NF- κ B, the NAD-dependent protein sirtuin deacetylase (SIRT1), and the sex hormone receptors ²⁵⁷. The high fiber content exerts indirect effects through the proliferation of commensal bacteria that reduce systemic inflammation through their interactions with the mucosa-associated lymphoid tissue and the generation of regulatory T cells ²⁵⁸. Healthy diets are also associated with high consumption of monounsaturated and polyunsaturated fatty acids that, when metabolized, generate resolvins and other anti-inflammatory ligands of the receptor peroxisome proliferator activated gamma (PPAR- γ), Toll-like receptors (TLRs), G protein-coupled receptor 120 (GPR120), COXs, and LOXs with antagonism in the activation pathways of NF- κ B ²⁵⁹. The ingestion of diets rich in complex carbohydrates and polyunsaturated fatty acids decreases the levels of insulin, IGF-1, estrogen, testosterone, and proinflammatory cytokines such as TNF α , IL6, and CRP ²⁴⁷. In addition, the low circulating levels of

biomarkers of inflammation are associated with a delay in the onset of all chronic diseases ²⁶⁰. Adherence to healthy diets shows lower levels of biomarkers of cellular aging ²⁶¹, hyperplasias ²⁶², prevents EMT, and delays tumor progression ²⁶³. Overall, healthy dietary patterns modulate epithelial carcinogenesis by slowing the rate of cell growth, proliferation, metabolism, and systemic inflammation. In doing so, they comprehensively delay aging, the accumulation of senescent cells, tissue infiltration, and inflammatory levels, resulting in a low probability of cellular transitions (Fig. 5.).

In contrast, obesity and unhealthy diets promote higher circulating levels of proinflammatory cytokines, sex hormones, insulin, and growth factors ²⁶⁴. An unhealthy lifestyle is associated with obesity and the early onset of chronic diseases ²⁶⁵. It is currently considered that obesity and western diets promote accelerated aging ²⁶⁶. Moreover, obesogenic diets promote the accumulation of senescent cells ²⁶⁷, induce EMT and tumor progression ²⁶⁸. In the case of infections, it is proposed that the etiological agents age and induce inflammation in some specific tissues since the process is mediated by receptors. Therefore, only the affected tissues increase cellular turnover and immune responses because of infection. Thus, the human papillomavirus, *Helicobacter pylori*, and schistosomiasis generate carcinogenesis of the cervical, gastric, and bladder epithelia, respectively. In the case of smoking and alcoholism, a similar event occurs, since due to the location some tissues are more exposed, or due to their activities in processes of detoxification are also highly affected. This fundamentally explains the preferential carcinogenesis of the lungs and liver respectively, and the association of both with oropharyngeal cancer.

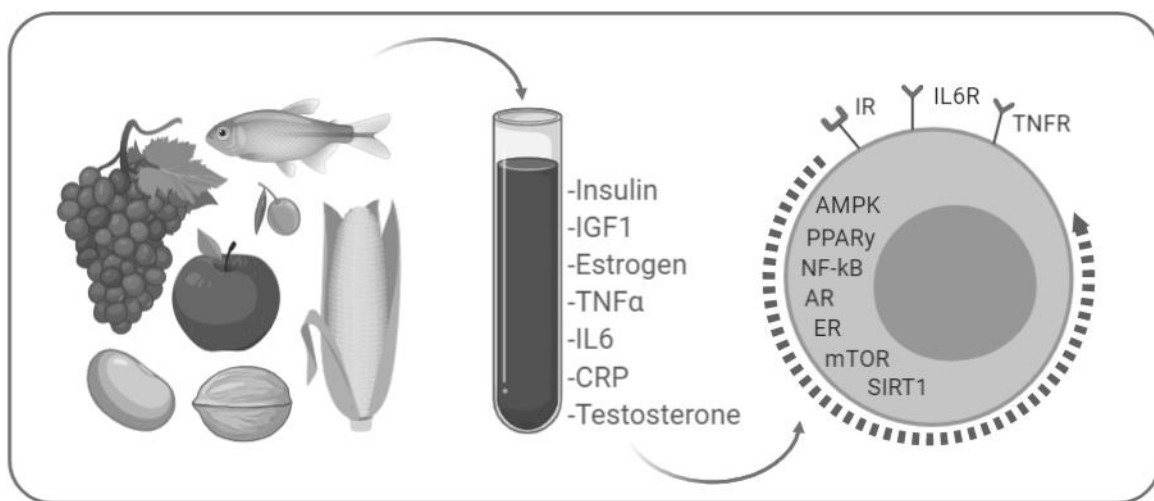


Fig. 5. Healthy diets modulate carcinogenesis by slowing cell growth, proliferation, metabolism, and inflammation. Blood biomarkers reflect the systemic response to diet. These studies indicate that a healthy dietary pattern induces low levels of insulin, IGF-1, estrogen, testosterone, and proinflammatory cytokines such as TNF α , IL6, and CRP. Chronic exposure to low levels of these ligands results in low activity of the IR, the mTOR protein, the ER, the AR, Ob-R, and cytokine receptors, and their signaling pathways. In general, healthy dietary patterns reduce the accumulation of senescent cells and inflammation of the tissues, so the conditions for the cellular transitions involved in epithelial carcinogenesis are not favorable.

In this theoretical framework, the effect of lifestyle on carcinogenesis converges in the modulation of aging and inflammation. It proposes a mechanistic and integrative overview of the most important regulators of the process considering the premises of the model. In conclusion, seem that a healthy lifestyle delays cellular aging and downregulates systemic inflammation resulting in cancer prevention, but ultimately it increases lifespan by slowing the process of development. In the following section, this type of view is transferred to cancer therapy in an effort to explain its failure along with an alternative that arises from the hypothesis.

8.2. Cytotoxic therapy promotes cancer.

Surgery is the central component of the curative treatment for localized solid tumors²⁶⁹. Cancer *in situ* is curable using surgical resection as the only therapeutic strategy⁵⁻⁸. Regarding early-stage invasive carcinomas, the contribution of surgery to the relative survival rate at 10 years surpasses 90%²⁷⁰. Therefore, several studies suggest that most of the beneficial effect of cancer therapy is attributed to the removal of tumor burden^{269, 271}. Although the 5-year relative survival rate of patients diagnosed and treated with low-grade carcinomas is greater than 90%, they eventually progress to high-grade carcinomas and die from causes associated with cancer^{37, 272}. The invasive metastatic cancers are responsible for 90% of cancer mortality⁵⁻⁸ and the only therapeutic option at this stage, due to relapses or tumor progression during treatment is palliative systemic therapy. In other words, surgery is not part of the treatment, only chemotherapy, and radiation. The use of cytotoxic therapies at advanced stages for palliative purposes or to increase survival is at a debate since high-grade tumors and metastases do not respond. However, the use of aggressive antineoplastic treatments is frequent in terminal patients despite the lack of therapeutic response. The net result is an increased cost and a reduction in the quality of life without a clear benefit for patient survival²⁷³. In contrast, a growing number of studies indicate that surgical resection of the primary tumor and larger metastases improve survival even in patients with high-grade metastatic cancers^{269, 274}. This suggests that most of the benefits of cancer treatment are derived almost exclusively from the removal of the cancerous tissues.

Historically, the therapeutic failure in cancer has been attributed to late diagnosis. In other words, tumors are detected when they already acquired most of the molecular mechanisms necessary to resist the treatment. However, studies have shown that resistance, metastasis, and aggressive behavior arise relatively quickly²⁷⁵. Lack of tumor response to treatment may occur from the first therapeutic regimen or after a temporal response²⁷⁶. Therapy is usually a combination of cytotoxic drugs and radiation to avoid the chances of developing resistance. However, they fail to eradicate cancer cells, and patients often relapse within months to a year after treatment. Furthermore, recurrences generally manifest a greater degree of malignancy and do not respond to therapy^{277, 278}. Tumor progression in the presence of therapy is traditionally understood by Darwinian mechanisms of positive selection mediated by the acquisition or modification of genetic traits. The hypothesis of cancer stem cells was recently added to explain treatment failure and tumor recurrence. This position considers that a small subset of cells in the tumor possess this cellular phenotype, the ability to self-renew and differentiate into tumor cells. Hence, they would be inherently resistant to cancer therapy and responsible for tumor recurrence²⁷⁹. Like many other phenomena in cancer, the origin of cancer stem cells and their role in carcinogenesis is not fully accepted. However, the last decade has validated the ability of chemotherapy and radiotherapy to induce stem cells within tumors^{2, 280-282}.

Most of the antineoplastics drugs, including the alkylating agents, antimetabolites, antimitotics, antibiotics, and epipodophyllotoxins are carcinogenic since they induce malignant tumors and secondary cancers^{7, 283}. The generation of neoplasms or the progression towards aggressive behavior has also been documented for radiotherapy^{216, 284}, and targeted therapies such as bevacizumab²⁸⁵ and imatinib²⁸⁶. Most of the tumors induced by cytotoxic therapy are acute myeloid leukemias, followed by solid tumors of the breast, bone, lung, prostate, ovary, bladder, and brain^{7, 283, 287}. The use of neoadjuvant chemotherapy (applied before surgery) also seems to promote tumor aggressiveness and recurrence negatively affecting survival²⁸⁸. One of the responses of the tumor to therapy is an increased transcriptional signature of embryonic stem cells^{118, 216}. In this regard, recent evidence indicates a relationship between senescence, EMT, and increased expression of embryonic genes with events triggered by antitumor strategies^{118, 289, 290}.

The hypothesis of epithelial carcinogenesis as the product of cellular transitions promoted by cellular aging and inflammation suggests a mechanism by which the current treatment is not only ineffective but accelerates cancer progression. Molecular responses to the non-lethal adverse conditions imposed by treatment promote

transcriptional derepression, inflammation and enable the emergence of undifferentiated cell states endogenously resistant and associated with a negative prognosis. Under this view, current cytotoxic therapies are doomed to failure in their attempts to cure cancer. According to the postulates of the hypothesis, the detrimental effects of cytotoxic therapy are mediated by the increase in the systemic load of senescent cells that translates into inflammation and accelerated cellular aging. Consequently, the EMT and events of dedifferentiation are promoted, increasing organ fibrosis and the emergence of cells with malignant and metastatic behavior that are stabilized in inflammatory conditions.

Current cytotoxic therapy leads to systemic events that promote accelerated tumor progression. Most patients exhibit transient pancytopenia caused by extensive damage to the bone marrow components^{291, 292}. These histological changes provide the basis for the generation of leukemias in response to cytotoxic therapy. In the first phase, the decrease in tumor burden and proinflammatory cytokines generate the partial or complete, but always temporal responses associated with cancer therapy. However, in a second phase, the continuation of the therapeutic regimen increases cell damage, senescence, dysbiosis, fibrosis, and the functional loss of many tissues. The cytokines produced by all damaged somatic cells, would overcome the initial reduction that manifest as cachexia. From here, the systemic conditions would only favor accelerated progression.

The antineoplastic drugs cyclophosphamide, fluorouracil, cisplatin, methotrexate, adriamycin, and etoposide induce cachexia^{293, 294}. The drivers of cachexia are the cytokines TNF α , IL6, IL1, CRP, and TGF β . The elevated levels of these cytokines generate insulin and IGF-1 resistance, which hinders the metabolism of glucose and amino acids in the skeletal muscle while favoring lipolysis in adipose tissue and the oxidation of fatty acids. The metabolic state is reminiscent of fasting but in feeding conditions. For this reason, the syndrome of cachexia is characterized by the wasting of the skeletal muscle and adipose tissue despite the presence of an adequate caloric intake. As cachexia progresses, cancer patients deplete the reserves in adipose and muscle tissue, generating weakness, motor difficulty, and cardiopulmonary dysfunction²⁹⁵. Cachexia is found in 80% of late-stage cancer patients, frequently along with metastasis and unresponsive tumors. Furthermore, those patients usually cannot tolerate the side effects of cytotoxic therapy. Their involuntary weight loss is accompanied by immunosuppression, lack of wound repair, fibrosis, and systemic dysfunction, which decrease their quality of life and negatively affect the prognosis. It has been estimated that cachexia is responsible for 40% of cancer death²⁹⁶. As expected, the use of parenteral nutrition or diets with calorie surplus is ineffective against the effects of cachexia. In the advanced stages, the secretions of the tumor masses are sufficient to generate cachexia. Proinflammatory cytokines reprogram the metabolism of the hosts for the mobilization of nutrients towards the tumor masses. Overall, those inflammatory and metabolic mechanisms increase tumor progression at expense of the host²⁹⁵.

The physiological conditions by which tumor nutrition is favored remarkably resemble embryonic development. During the second half of gestation, the maternal reserves are mobilized to allow the fetal exponential growth. The production of glucose, amino acids, and fatty acids in the bloodstream is increased due to insulin resistance, gluconeogenesis, proteolysis, and lipolysis. These effects are mediated by insulin, IGFs, glucagon, catecholamines, cytokines, and leptin. Insulin resistance in the mother ensures proper sparing of nutrients and energy to the fetus to fulfill the anabolic pathways since its metabolism is relatively independent of the activity of insulin. Also, the fetus overexpresses proteins related to the transport and metabolism of nutrients, among which glucose, lactate, ketoacids, amino acids, and fatty acids stand out. The anaerobic metabolism of these substrates is the main source of energy and growth²⁹⁷. All of the mechanism described has been considered the metabolic characteristics of tumors²⁹⁸. However, the reactivation of developmental events seems to naturally explain the metabolic process responsible for cancer cachexia.

Given the above arguments, the direct effect of cytotoxic therapy might result in a net increase in cellular transitions towards a mesenchymal stem cell phenotype. At the same time, therapy induces cachexia, which

indirectly favors the maintenance of the dedifferentiated phenotypes and their metabolic and migratory activities. Meanwhile, the nutritional and inflammatory status of the host is at disadvantage. The approach to cancer as a developmental disease provides a simple explanation for therapeutic failure in advanced stage carcinomas.

Since the 1970s, cytotoxic agents and radiation therapy have been an important part of the treatment of most low-grade carcinomas despite their little contribution to survival. Likewise, they are used in the palliative treatment of high-grade carcinomas regardless of their null effectiveness. The incorporation of drugs directed at molecular targets was irrelevant in survival since most signaling pathways are highly redundant and the complete blockade of essential elements is lethal for the host ²⁹⁹. Hence, for the most part, high-grade cancers, remain untreatable and incurable diseases ²⁷¹. Furthermore, since the first requirement in the guideline for the search for new anticancer agents is the cytotoxicity at very low concentrations ²⁹⁹ is foreseen that during the next years the new marketed molecules as antineoplastic drugs, will suffer from the same problems as the current agents, essentially, they will be very toxic and fail to cure cancer.

8.3. Paving the way for tumor reversion.

Countless experiments demonstrate the ability of tumor cells to differentiate. The first observations that tumor cells could originate a normal tissue come from studies carried out with teratomas in 1907 ³⁰⁰, while the regression and differentiation of an epithelial tumor were possible in 1962 through its implantation in normal tissue ³⁰¹. However, tumor differentiation with a pharmacological agent was possible until 1988. All-trans retinoic acid (ATRA) allowed complete remissions in cases of acute promyelocytic leukemia (APML) by inducing terminal differentiation of leukemic cells to granulocytes. With this type of treatment, more than 75% of patients diagnosed with APML are cured. The implementation of differentiation therapy in leukemias seems logical since many of them are characterized by the accumulation of immature lymphoid cells ³⁰². However, in the rest of the neoplasms, the genocentric view of cancer conceptually weakens the therapeutic potential of induction of differentiation in at least two fundamental points. It does not consider that solid tumors are generated by immature precursor cells and postulates that cancer cells are unable to differentiate since possess an aberrant genetic code. The scarce events of cell differentiation in tumors were reconciled with the conventional genetic approach by attributing them to the activation of endogenous differentiation pathways that were unaltered during carcinogenesis.

On the contrary, in the hypothesis of epithelial carcinogenesis as a product of discrete cellular transitions, it is implicit that solid tumors are also originated from undifferentiated precursor cells. In addition, this approach allows the understanding of tumor reversion by inducing differentiation even in the presence of genomic damage. In the theoretical framework presented here tumor reversion is achieved through the induction of cell differentiation. In this view, the modulation of proliferation, senescence, and the inflammatory pathways with pharmacological agents modifies the epigenetic landscape to induce cell differentiation. The use of molecules from diverse chemical and structural nature can induce the attainment of a differentiated phenotype even in the absence of specific instructions (as would be the case of the stroma, of specific lineage growth factors, etc.) or conventional pharmacological interactions (Fig. 6.).

The objective of differentiation therapies in cancer is primarily to induce phenotypic maturation to decrease aggressive tumor behavior ³⁰³. Preclinical studies to assess the capacity of drugs to induce differentiation in solid tumors are scarce but are consistent with the premises of the model. For example, the use of troglitazone in patients with liposarcoma, demonstrated histological changes consistent with differentiation into adipocytes ³⁰⁴ and the use of ATRA in nasopharyngeal carcinoma increases the expression of epithelial markers ³⁰⁵. The molecular mechanism of both events of differentiation converges in the regulation of NF- κ B and upregulation

of transcription factors that induce the normalization of tumor tissues towards organized histology that expresses markers of differentiated cells^{304, 305}.

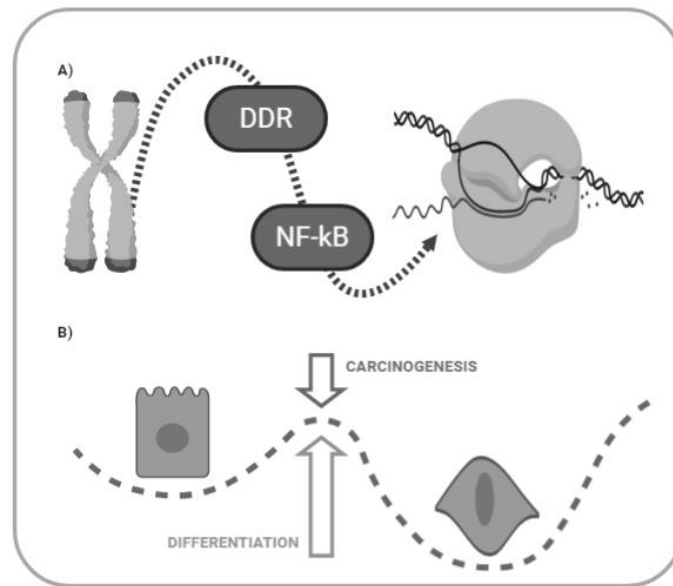


Fig. 6. Mutationless carcinogenesis, differentiation in absence of instructions.

The DNA acts as a robust network to structural changes, that is, most mutations do not affect the epigenetic landscape. Cells can differentiate into normal phenotypes despite mutations, and cancer can be induced in the absence of mutations. A) Mutations or genomic damage do not confer malignant traits, but rather activate and perpetuate the DDR and cellular inflammation (NF-κB), causing a significant loss of the mechanisms of transcriptional repression. B) This leads to the modification of the epigenetic landscape and allows the affected cells access to a multipotent state with a transcriptional profile of embryonic cells and the potential to induce cancer. Most carcinogens converge on these mechanisms, including cellular senescence. Telomere attrition facilitates the acquisition of chromosomal damage, which is established as DDR, cellular inflammation, and increased transcriptional activity. Proinflammatory cytokines and EMT also allow access to a transcriptional state with a configuration similar to the mesenchymal stem cell. Therefore, nonspecific mutations allow access to the cell state that causes cancer. Similarly, ligands of diverse structural and chemical nature can lead to cell differentiation by altering the epigenetic landscape and essentially reducing cellular inflammation.

The induction of structural and functional changes in cancer cells related to phenotypic maturation is called cytoeducation³⁰⁶. The study of the mechanism underlying the reversion of the malignant phenotype in cell cultures is growing and among the molecules with this type of effects is the ATRA^{307, 308}, 1 α , 25-dihydroxyvitamin D3¹⁹, the coumarins daphnetin and 5-methoxy-6,7-methylenedioxycoumarin^{309, 310}, the flavan-3-ols, such as catechin and epicatechin, and the flavonoids wogonin and apigethrin³¹¹. The exposition of cancer cells to these compounds causes the inhibition of the cell cycle and the cancer-associated signaling pathways, including NF-κB³¹¹. Is expected that the elucidation of the mechanisms by which some agents induce the differentiation of cancer cells eventually allows the establishment of more rational strategies for the treatment of cancer. This approach will lead to the development of less toxic agents to improve the organization of the affected tissues and the expression of epithelial markers. This would guarantee a delay in progression accompanied by an increase in survival. Based on the same rationale, the optimization of this type of therapy requires the surgical removal of tumor masses, the use of antiinflammatory and antiproliferative agents in conjunction with a healthy lifestyle to increase the chances of tumor reversal. We hope that the implementation of this type of strategy for cancer treatment will lead to an increased rate of remissions and eventually the chronic management of the disease.

9. Conclusion

Through the studying of epithelial carcinogenesis was developed an integrated theory that reconciles disperse results in an evolutive and developmental framework of the underlying causes of cancer. The rationale of the mechanistic association of aging and inflammation with sporadic carcinomas was uncovered and suggests the basis for the study of chronic diseases as part of the developmental process. The hypothesis of epithelial carcinogenesis as the result of cellular transitions promoted by cellular aging and inflammation considers cancer an endogenous cell state that matches the transcriptome of stem cells. Proposes that sporadic carcinomas are mainly the result of the reactivation of developmental processes in hyperplasias and implies that epithelial cells undergoing replicative senescence are susceptible to reprogramming *in vivo* using cellular transitions promoted by inflammation. These transitions are driven by transcriptional derepression that enables stemness, healing, and cancer. Although it might seem controversial, it provides a natural explanation of paradoxes accumulated in the field, and most importantly a rational basis for the therapeutic failure in cancer. The result of this theoretical endeavor offers a simple model to capture knowledge from the ever-increasing complexity in the molecular and cellular biology of cancers. Derived from the insights of the hypothesis the role of lifestyle and therapy was critically reviewed and some encouraging strategies for the management of carcinomas are provided. In conclusion, the underlying order in carcinogenesis suggests that is a disease resulting from the process of development and its interplay with lifestyle.

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