Artykuł przeglądowy *Review*

Ambivalent impact of senescent cells on the proliferation of neoplastic cells

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Summary

The study shows that carcinogenesis in old age occurs through a direct pathway, i.e. through the action of mediators excessively produced by senescent cells on subsequent stages of oncogenesis, and/or through an indirect pathway. The second possibility occurs through the aforementioned mediators involved in the process of aging-related inflammation (inflammaging). In this reaction, immune system cells are also recruited by pro-inflammatory substances secreted by senescent cells. Aging-related inflammation has many features of "internal inflammation" without an infectious factor and can induce cancer, and conversely, cancer induces internal inflammation as a response to its action. A key role in this process is attributed to the tumor microenvironment (TME) on its progression and vice versa. Mutations in the cell's environmental genes (landscape genes) contribute to the creation of conditions for uncontrolled proliferation of these cells. The paper also presents senescent cells in the process of evolution, emphasizing the fact that evolution has not eliminated cancer, as they are probably the only structures that guarantee cellular "immortality". Cancer, therefore, is likely a structure corresponding to infinite ("immortal") fractures.

Keywords: aging cells, senescent cells, aging-related inflammation, cancer induction, cancer evolution

Somatic cells age in two types of processes: 1 – replicative senescence SASP (senescence-associated secretory phenotype), conditioning final cell cycle arrest due to p53 and p21 protein activity decline, which for highly proliferative cells means 40-90 telomero-dependent divisions or p16 protein for cells of lower activity (5-20 also telomere-dependent), as well as mosaic senescence (telomere-dependent or telomere-independent depending on the cell type), and 2 – stress-induced premature senescence (SIPS), telomere-independent (1-5 cell division), involving p19, p21, p27, p53 proteins (45). The second type, although it applies to normal somatic cells, might also apply to the neoplastic cells, which is of heightened interest in most recent oncology. When in replicative senescence the main underlying cause of DNA damages is reactive oxygen species (ROS), belonging to free oxygen radicals, and nuclear targeting signal (NOS), followed by ATM and CHK/2 kinase activation, NBS1 protein, NF-κB transcription factor, p38 protein kinase activated by p38MAPK mitogens and inflammasomes, the oxidative stress leads to DNA damaging due to retrograde signaling response meaning increased mitochondrial biogenesis, being followed by overproduction of ROS, as a reaction to cellular ATP reserves decrease (1, 37). In senescence, genetic factors are direct cells from the replicative state to a non-replicative state, therefore it is not a simple random phenomenon (37, 38). Replicative senescence reduces neoplastic proliferation, where the only limitation are telomeres, shortening with every chromosome division, due to telomerase activity. The high level of this enzyme is present in most malignant tumors, which correlates telomerase reactivity and *N-myc* amplification, promoting cell immortality (44). Telomerase reactivity breaks the bridge-fusion-breakage (BFB) cycle of the cell avoids mitotic catastrophe and apoptosis (41). Similar phenomenon happens with Bcl-2 and IAPs proteins

inhibiting apoptosis overexpression (41). Telomeres that are too short lead to genetic defect accumulation, ex. chromosome fusion or translocation, which inhibits immortal process in neoplastic cells. In this situation even suppressor genes (*TP53*, *RB1*) mutations and proto-oncogenes coding genes (*ras*, *myc*) cannot prevent immortality of those cells (10). This explains why Amish people in the USA, living up to 90 years old, have prolonged telomeres by 10% (genetic mutation), and long-lived lobsters do not shorten telomeres during cell division. Paradoxically, telomerase inhibition might simulate oncogenesis, which has been observed in congenital dyskeratosis, where dyskeratin mutated gene binds with RNA, destabilizing telomerase, leading to increased susceptibility for malignant tumors (45). Oxidative stress is sometimes accompanied by epigenetic, metabolic and replicative stress, but also endoplasmic reticulum (ER) stress that lrads to incorrect protein folding in the cell subsequently causing apoptosis (10, 11, 14). Oxidative stress might also influence the speed of teomeres shortening (25). It might also inactivate anti-proliferation PTEN phosphates (cancer suppressor possessing the *PTENP1* pseudogene encoding lncRNA) action, which increases Akt/PKB and SGK kinase activity. PKB/Akt is anti-apoptotic throughout NO and ROS synthesis stimulation (32).

The decision of cell survival or senescence depends on the balance between Bcl-2/Bax. Senescence of ageing cells, as well as neoplastic cells, might be paradoxically inhibited during apoptosis, when both cell types do not enter the cell death pathway, meaning that ageing cells as well as neoplastic cells are resistant to apoptosis (5). It needs to emphasized that at the same time caspases are activated in neoplastic cells, whereas normal cells have non-activated pro-caspases, meaning the first are more likely to revealing apoptosis than normal cells. So caspase action is regulated differently in neoplastic cells than normal cells (29). This is sometimes accompanied by impaired efferocytosis, which is the removal of apoptotic bodies by phagocytes (monocytes, differentiated macrophages, CD11c+ dendritic cells). These bodies, in the final phase of apoptosis, contain fragments of the disintegrated cell nucleus and, before being phagocytosed, may likely transmit characteristics of mutated material in the remnants of chromatin (45). A similar situation is observed in chromotripsy of chromosomes, i.e. a one-time event of "cutting" chromosomes followed by tens or hundreds of genomic rearrangements (29). Therefore, it is assumed that senescent cells may initially act as inhibitors of oncogenesis. An example of such a process is the SASP phenotype, which can act ambivalently, i.e. both inhibiting and stimulating cancer development. The secretory phenotype of the senescent cell may initially be responsible for inhibiting the cell cycle in neighboring cancer cells through juxtacrine signaling (cell adhesion molecules, TNF cytokines, EGF, Hedgehog), as the reception of information from the signaling cell occurs solely through direct contact with the target cell (20). However, the presence of cytokines in the late SASP destroys cancer cells as a result of the immune response, although as a side effect, this phenomenon can sometimes lead to the stimulation of tumor proliferation (immunoediting), which is expressed by increased angiogenesis, the formation of cancer metastases, and the suppression of the immune response (44, 45). Therefore, it is now assumed that a short-term and intense SASP process acts as a suppressor, while a chronic process usually activates tumor proliferation. Regardless, after prolonged expression, the regeneration process becomes dysregulated, which also favors oncogenesis.

The cell cycle is activated/inhibited by the mTOR protein (mammalian target of rapamycin). Through the mTORC1 complex, mitochondrial DNA (mtDNA) can undergo mutations, leading to mitochondrial dysfunction, oxidative stress, mutations, and oncogenesis (19, 44). Inhibition of mTOR slows down, among other things, aging-related inflammation, associated cancers, and autophagy.

A significant role in carcinogenesis is also attributed to cancer stem cells (CSCs), which originate from hematopoietic stem cells (HSCs) of the bone marrow, from which cells other than hematopoietic can also arise, as the gene expression pattern of a differentiated cell is not permanent, as evidenced by the cloning of an entire organism from the nucleus of a mammalian cell (Dolly the sheep (29)). Thus, CSCs can initiate cancer, which is caused by errors in DNA replication and the accumulation of mutations greater than in other cells (26). Furthermore, the presence of multipotent stem cells known as BASCs in the lungs of mice has been demonstrated, which not only have the ability to regenerate damaged cells, but also, when exposed to an initiating hit, such as a somatic *K-RAS* mutation, allow these cells to bypass "checkpoints" in the mitotic cycle and induce adenocarcinoma (cit. from 10). Inhibition of CSC differentiation results in an increase in their number, as observed in leukemia in humans, where these cells express the CD34 antigen and lack expression of the CD138 antigen (40). Leukemic stem cells (LSCs) represent 0.1-1% of blasts, have low proliferative activity, and are associated with the bone marrow microenvironment (niche). They are capable of surviving intensive therapy and may then form a pool of cells that are the source of disease remission. In such cases, numerous new mutations occur, leading to significant clone heterogeneity and a longer survival time for these cells (45). These cells can also form a residual pool of leukemia cells (so-called minimal residual disease – MRD), which is undetectable without the use of flow cytometry or molecular methods.

Longer survival time of cancer cells, rather than increased proliferation, applies to, for example, cells of chronic lymphocytic leukemia and small lymphocytic lymphoma – CLL/SLL – B cell chronic lympho-

cytic leukemia/small lymphocytic lymphoma, which is caused by the accumulation of a large amount of BCL2 protein, an anti-apoptotic factor. As a result of chromosomal deletions, there is over expression of BCL2 and the loss of genes encoding miRNA, i.e. negative regulators of this protein (1). The possibility of multiple stem cell lines with diverse characteristics is also considered, as well as the appearance of new cells, particularly in cancer progression (45). It has also been observed that normal stem cells, during division, can acquire unpredictable mutations and accumulate them, thus becoming a potential source of carcinogenesis, for example, migrating to sites of chronic inflammation (29). In elephants, it was noted that pluripotently induced stem cells (iPSCs) contain the Oct4 protein involved in the self-renewal of undifferentiated embryonic stem cells, nuclear DNA, and the actin protein. These animals rarely develop cancer, as they have as many as 29 types of *TP53* genes, instead of the one found in other mammals, which protect the p53 protein from uncontrolled growth.

Old cells themselves cannot undergo cancer transformation due to the completed ability to enter the division cycle. They thus become an "energy burden" for the organism, which eventually leads to their atrophy, fibrosis, or necrosis. Furthermore, senescent cells disrupt the function of other cells, inhibit stem cell function, trigger fibrinolysis, and organize the removal of damaged or potentially oncogenic cells (10). At the same time, these cells can participate in inhibiting oncogenesis; for example, by impairing the morphogenesis of epithelial cells in the mammary gland in women, inhibiting the development of breast cancer, and also playing a role in tissue regeneration and wound healing. Moreover, senescent cells, "not wanting to die", may produce an excess of mediators with properties that stimulate the proliferation of other cells and/or, through "sterile" senescent inflammation, participate in cancer transformation (Fig. 1). Such mediators include cytokines (IL-1, IL-6), pro-inflammatory chemokines (CCL2, CXCL1, CXCL8), growth factors (EGF, FGF, GHF, VEGF, heregulin), proteolytic enzymes and their inhibitors (MMP-1, 3, 10, TIMP-2, PAI-1, t-PA, uPA), adhesion molecules (ICAM-1, VCAM-1), receptors (sTNFR1), as well as ECM (extracellular matrix) elements such as collagen and fibronectin (45, 47). Sometimes, one mediator can stimulate the release of other mediators, often intermediates, with ambivalent effects, i.e., either enhancing or inhibiting the action of the initial mediator (10). These mediators stimulate further stages of cancer progression, i.e. adhesion → proliferation → migration → invasion → EMT (epithelial to mesenchymal transition) → MET (mesenchymal to epithelial transition), as well as angiogenesis, and participate in senescent inflammation present in the aging process (9, 19, 27, 35). The first possibility can therefore be considered a form of "indirect" oncogenesis, while the second is a "direct" oncogenesis. Regardless of the aforementioned causes, as the organism ages, DNA repair mechanisms (DNA damage response) fail, which, given the total length of this nucleic acid in humans, around 20 million kilometers, is not uncommon, and a mutated cell proliferates spontaneously and undergoes cancer transformation (1, 4). Furthermore, aging

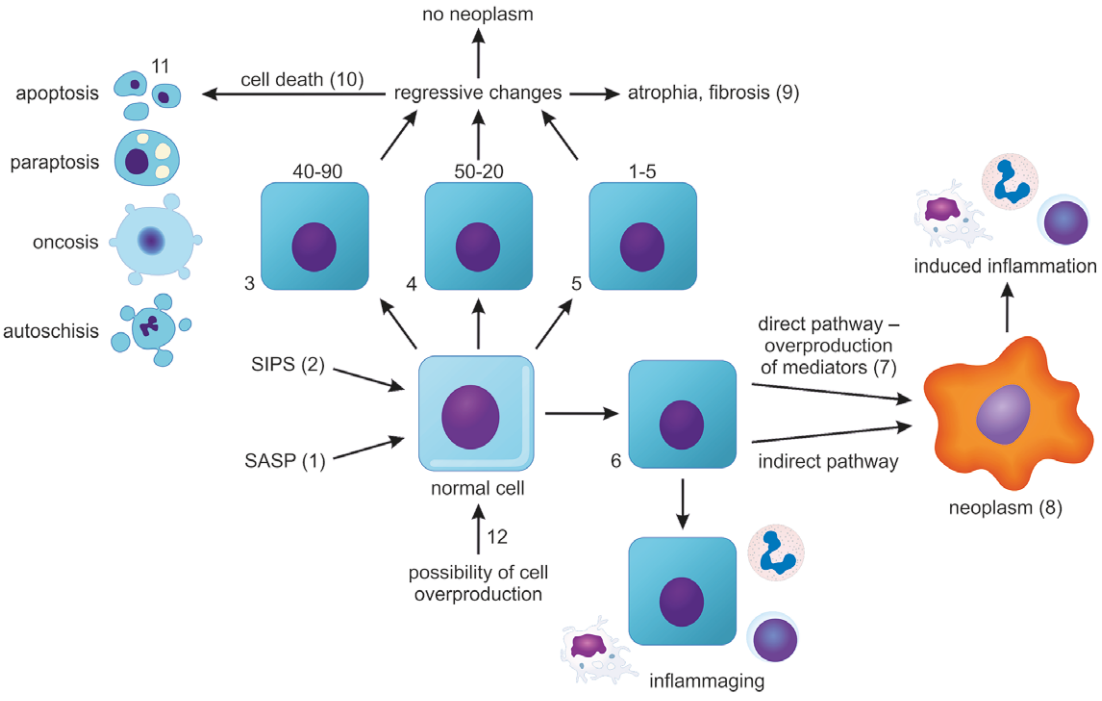

Fig. 1. Ambivalent role of senescence cells in tumor transformation
Explanations: 1 – senescence-associated secretory phenotype (SASP), telomere-dependent; 2 – stress-induced premature senescence (SIPS), telomere-dependent (with small exceptions), mainly affecting young cells; 3 – senescent cells after 40–90 divisions, telomere-dependent; 4 – senescent cells after 50-20 divisions, telomere-independent; 5 – senescent cells after 1-5 divisions, telomere-independent; 6 – non-dividing senescent cells; 7 – stimulating cell proliferation; 8 – intermediate tumor transformation; 9 – increased levels of transforming growth factor beta 1 (TGF-β 1), stimulating collagen synthesis; 10 – necrosis is preceded by an agonal state of the cell, which activates genetic programs of apoptosis, parapoptosis, oncosis or autoschisis; 11 – apoptosis is hormonally regulated, influenced by growth factors, and cytokines; while necrosis is not and once initiated leads to cell death; 12 – in the process of cell replication, the excess cell must die or become a cancerous cell, e.g. as a result of mitotic catastrophe

leads to the involution of the thymus and a decline in the number of naïve T lymphocytes, a reduction in the ability to recognize foreign antigens, and rearrangements of these cells' genes. This results in the formation of circular DNA molecules (so-called TREC – T-cell receptor excision circle), whose number decreases with age (1).

A significant influence on oncogenesis also comes from the tumor stroma, as the production and degradation of the ECM accompany tumor invasion and metastasis, as well as the entire network of molecular connections, which accumulate, rather than, as previously assumed, a change in the signal on one of the pathways, such as *K-RAS* mutation, compartment unlocking by mutation, or apoptosis inhibition by *TP53* mutations (4, 11, 17). Moreover, stromal cells release TGF-β, IL-6, and matrix metalloproteinases (MMP-1); these are activated in the vicinity of tumor cells, facilitating their movement and conditioning angiogenesis (3, 6, 7). On the cell membrane of tumor cells there is positive regulation of the protein, the inducer of extracellular matrix metalloproteinase (EMMPRIN), which triggers MMP in the vicinity of stromal cells (28). Stromal cells also produce growth factors in the ECM and indirectly activate proliferation, such as insulin-like growth factors associated with binding proteins (IGFBP) of tumor cells and stimulate their mutations, prompting these cells to further divide and survive (45). Mesenchymal stem cells (MSC), present in the inflamed tissue, are activated and secrete chemokines and regulatory cytokines (TGF-β, IL-6, PGE2), growth factors, induce iNOS or iDO activity (indoleamine-2,3-oxygenase – an enzyme overexpressed in tumors causing immunosuppression), and secrete extracellular vesicles (1). The inflammatory process can also be potentiated, for example, in the phase of immunological equilibrium, by B lymphocytes inhibiting Tc lymphocytes, which stimulates tumor development. Regardless of this, senescent inflammation bridges two similar processes occurring in both oncogenesis and atherosclerosis, such as the participation of M1 macrophages (pro-inflammatory, anti-tumor) and M2 macrophages (anti-inflammatory, pro-tumor) in both processes, atherosclerotic inflammation of blood vessels, and in cancer, a chronic inflammatory reaction around the tumor (5, 31, 37). It should also be mentioned that the tumor microenvironment acts immunosuppressively through factors released by ECM cells (TGF-β, IL-10) and factors secreted by tumor cells (adenosine, TGF-β, IL-6, IL-6, VEGF). This triggers an immune response in the form of T-cell immunity directed against the tumor (45).

Recently, particular interest has been given to the EMT transition, a process dependent on the activity of histone methyltransferase, in which cancer cells undergo stages of the early phase of the EMT hybrid → EMT hybrid → late phase of the EMT hybrid → mesenchymal tumor cells (23, 29). Moreover, EMT generates cells with stem cell characteristics. In such a situation, inflammation can recruit these cells from the bone marrow and participate in changes in the phenotype of tumor cells. This has been proven using mesenchymal stem cells (MSC) in prostate cancer in humans, as a response to the chemoattractant CXCL16, followed by EMT promotion and cancer invasion (26, 46).

The overproduction of mediator substances by senescent cells, such as fibroblasts or mesothelial cells of the peritoneum, most likely stimulates, both *in vivo* and *in vitro*, various stages of tumor progression in cells capable of division: as mentioned earlier, these first cells are devoid of this ability. Thus, senescent cells are not directly involved in tumor transformation but indirectly, through metabolic processes (Fig. 1). It is also known that senescent cells behave differently, i.e. they are capable of inhibiting the tumor process. This likely explains the decreased incidence of cancer in very late old age, due to the maximal increase in the number of senescent cells during this period. Unfortunately, in such situations, further stimulation of tumor proliferation can occur, due to enhanced angiogenesis, the formation of cancer metastases, as well as the suppression of the immune response. Interestingly, the aforementioned cells age faster in patients with type 2 diabetes, in whom cell aging contributes to the development of late-life diseases, while simultaneously being one of their consequences (the existence of a vicious circle (19, 45, 46).

Senescence cells (SCs) act both stimulatory and inhibitory (ambivalently) on tumor cells (TCs), which can be graphically represented in the form of mathematical Euler circles (24) (Fig. 2). These circles illustrate the scope of interaction between the two aforementioned cell types, i.e. SCs and TCs.

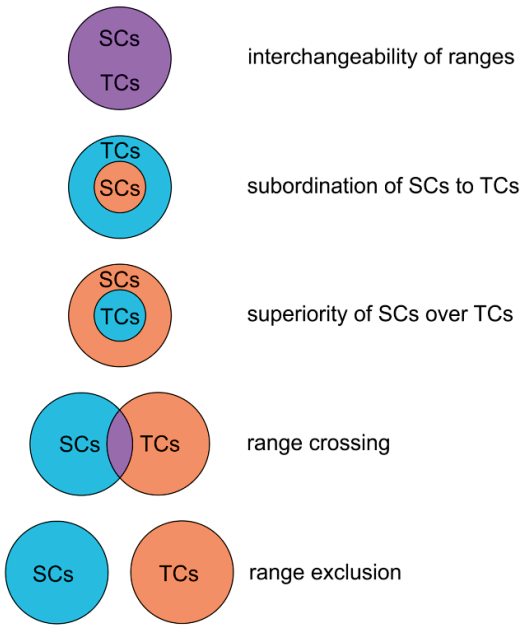

Fig. 2. Relationship between senescent cells (SCs) and tumor cells (TSs) presented with Euler's circles (24)

Proliferation in cancer, similar to SASP, is limited by replicative senescence due to telomerase (telomere-dependent aging), as well as *in vitro* – mortalin (a heat shock protein HSPs – mthsp 70/PBP74/GR 75), as described in an earlier study (14). It also presents the ambivalent effect of protein p53 and the *TP53* gene on this process. This gene protects young cells from oncogenesis, but with age it becomes one of the effectors of cell aging (45). This is an example of the antagonistic pleiotrophy of this gene.

Aging is programmed – DPS (developmentally programmed senescence) – while oncogenesis is the opposite or very rare. It is even assumed that a tumor represents a local "rejuvenation" of tissues, i.e. a regression of oncogenesis compared to senescent cells. An example of this can be the changes in cancer cells under the influence of premature aging induced by stress. Aging of somatic cells occurs, as mentioned earlier, at different times: 1 – after 40-90 divisions; 2 – 5-20, or 3 – 1-5 during SISP, for example, in cancer cells, depending on the activity of inhibitory proteins (p19, p21, p27, p53) (1, 45). The theory of free radicals was considered until now as the cause of cell aging through oxidative stress and the overproduction of ROS. However, it has been shown that inhibiting this stress with antioxidants or genetic engineering of antioxidant enzymes did not extend the lifespan of complex organisms (44, 45). ROS participate in many physiological processes, such as oxidative burst, inflammation (NF-κB activation, cyclooxygenase), cell differentiation, glucose transport, signal transduction, and influence on endogenous antioxidant systems (2, 30, 42). This phenomenon is called hormesis, where low doses of toxic substances have positive effects (45). It has also been shown that in high concentrations, antioxidants act as pro-oxidants, increasing oxidation and protecting both healthy and cancer cells from damage, as well as generating more free radicals through the Fenton reaction.

Some senescent cells die, some, not "wanting" to die, become unnecessary energy burdens, and others produce an excess of substances stimulating cells to undergo cancerous proliferation. Thus, senescent cells lead to oncogenesis indirectly, as they can no longer enter the cell cycle and divide. Cells in which apoptosis does not occur may undergo cancer transformation, and in immune cells directed against their own body, autoimmune diseases may arise (4, 16). Furthermore, cancer cells must bypass the aging process, which limits the cell's ability to divide. Thus, both senescence and apoptosis may, to some extent, prevent oncogenesis, as observed in the spontaneous involution of congenital hemangiomas in children (9, 10). Therefore, oncogenesis is thought to stimulate cell aging as a protective response that aims to inhibit tumor development (45). The effect of senescent cells on oncogenesis, through the induction of chronic inflammation, is not the only path to cancer transformation (an indirect path), but most likely a process conditioned by a whole network of other phenomena that may dampen or enhance each other (37). It is therefore not a simple stochastic (random over time) process but a multi-pathway one, whose final effect may be unpredictable. Senga and Grose even believe that in tumor development, additional characteristics such as dedifferentiation/transdifferentiation, epigenetic deregulation, changes in the microbiome, and nerve signaling should be considered (33). Paul, on the other hand, emphasizes that we should not focus only on cancer cells themselves but on the systemic features of the tumor (27). A tumor is not exclusively an autonomous structure but one dependent on the interaction between cancer cells, the tumor microenvironment, and systemic signals (39, 40, 42).

Cell aging in the process of evolution

A bold hypothesis has recently been proposed suggesting that aging is an unintended genetic consequence of evolution (cited in 36). The aging process is accompanied by an inflammatory state induced by this process (inflammaging), resulting from a disrupted balance between inflammatory and anti-inflammatory processes (anti-inflammaging) (44). Evolution is not "concerned" with the aging of an organism but with the process of reproduction, which essentially aligns with the development of cancer (22). Just like the aging process, cancers most likely escape the control of genetic safeguards, whose main task is to protect reproductive cells, not somatic ones, to ensure the continuity of the species. In most animal species, the loss of fertility is associated with the onset of rapid aging, except in humans, certain primates, whales, and budgerigars. According to Ridley, when faced with the dilemma of reproduction or immortality, nature always chooses the former; thus, reproduction comes at the highest price – old age and death, meaning that evolution will never make us immortal, which excludes fertility (32). Reproduction ensures the continuity of evolution, as an inseparable competitor to old age and death (13, 35). This process becomes evident only in old age, e.g. in the form of neurodegenerative diseases of the brain (Parkinson's disease, Alzheimer's disease), as well as a sharp increase in cancer incidence, both in humans and animals, usually after the reproductive period. This is due to the lack of energy for regenerative processes in somatic cells, which causes their retrograde changes (atrophy, necrosis), aging, and death. According to the adaptive theory of oncogenesis explaining the evolution of cancer cells, these cells succeed relative to other cells when their extracellular matrix (ECM) is damaged, e.g. by aging-related inflammation or cancer-induced inflammation (6, 20, 37). This explains why, contrary to evolutionary plans, the extension of an organism's lifespan beyond its reproductive period has resulted in the emergence of the aforementioned aging inflammation, i.e. entropic (aging) inflamma-

tion. Aging can take the form of diseases resulting from aging, such as cancer or Alzheimer's disease. Aging is a byproduct of the expression of genes, whose regulation, according to current theory, is controlled by the so-called "dark matter" of DNA, which constitutes 98.5% of human DNA and contains no genes (1). This implies that non-coding DNA ensures the "architectural planning" of all living organisms (45). Furthermore, in non-coding parts of the genome, there are many genetic variants (polymorphisms) associated with various diseases (29).

Since genetic changes in cells are inherited, after cell division, the daughter cells will undergo Darwinian selection, with a focus on the survival of the fittest, the most aggressive cells (10). This process is continuous and constantly evolving. All "means" are allowed to avoid death and survive, even at the cost of living with mutations. For example, cancer cells must acquire traits that enable them to resist internal stresses (internal apoptosis pathway), and circulating tumor cells (CTCs) must become resistant to anoikis, meaning survival without attachment to the ECM, through EMT and the expression of specific oncogenes, such as EGFR (29). Could it be that evolution favors cancer, especially in light of the data suggesting that new mutations drive it, while aging is seen in some sense as the "reversal of evolution"? Evolution has not eliminated cancer because they are likely the guarantee of cellular "immortality". Immortality arises, among other things, from the fact that cancers are likely structures corresponding to fractals of the infinite ("immortal"), where the fractality of microcirculation is one of the main factors facilitating the intra- and extravasation of cancer cells, alongside turbulent blood flow, instead of laminar flow, and the shear forces of sticky blood (shearing forces) (12). Both primary tumors and their metastases have an infinite, unpredictable, constantly growing, and non-stochastic fractal (correlational) outline, which arises from their development, progression, and "immortality" (12).

According to Huxley, synthetic evolutionism (neo-Darwinism) agrees with the theory of natural mutations, according to which molecular evolution takes place largely outside the control of natural selection (20). It is currently accepted that mutation and Darwinian selection in malignant tumors can lead from their monoclonality to genetic heterogeneity, especially in the later stages of their development (10). As a result, these tumors become increasingly aggressive and resistant to therapy, leading to the host's death, and thus the death of the tumor. This is an unresolved contradiction of interests, although logical. This happens even in situations where the cellular metabolism shifts in favor of the tumor through the glycolytic pathway (Warburg effect), involving increased glucose uptake and conversion to lactate (fermentation) (26). This is accompanied by a newly recognized oncogenic mechanism, namely oncometabolism, in which, for example, the mutated enzyme isocitrate dehydrogenase (IDH) in the Krebs cycle of cancer cells produces the oncometabolite 2-hydroxyglutarate (2HG) (10). Other factors include the avoidance of apoptosis, mitotic catastrophe, cellular senescence, immune surveillance, and the acquisition of the ability to have unlimited replicative potential (immortality) by cancer cells.

The individual cure of an adult patient who has offspring (inheriting their genes) is a great success for the patient, medicine, and the genius of human reason. At the same time, this means that pro-cancerous genes or at least a "predisposition" to cancer have been passed on to the offspring. In this way, reproduction wins over natural selection – the primary feature of evolution. Evolution will always choose reproduction over caring for aging somatic cells, for which there will no longer be energy substrates. Why evolution has overlooked the elimination of pro-cancerous genes or the predisposition to cancer formation remains unknown. At the same time, the "immortality" of cancer cells is only possible as long as the host organism lives or is artificially maintained in vitro, such as HeLa cancer cells.

Genetic changes in cancer cells are inherited after their division, meaning they undergo Darwinian selection and continually evolve towards progression (malignancy) and therapy resistance (10). This results in the formation of subclones that are resistant to current drugs. The growth and loss of cancer cells in a tumor is not balanced, as usually only a portion of its cells divide, which is known as the growth fraction. The loss of cells occurs through apoptosis and necrosis. In such a situation, as seen in breast and colon cancer in humans, the increase in the number of cells is usually only 10% greater than the loss of cells; the opposite happens in lymphomas (45). This has significant implications for chemotherapy, where cytostatics primarily destroy cells that are actively synthesizing DNA. Therefore, successes in treating lymphomas, where the proliferating fraction is higher compared to cancers, and is only about 5% (45). At the same time, it should be added that genotoxic drugs that damage DNA may lead to the loss of apoptosis and the accumulation of mutations, which in turn increases the risk of carcinogenesis. Such a phenomenon occurs, for example, in therapy-related leukemia, where a new cancer develops after chemotherapy (38).

Summary

As cells age and various endogenous substances accumulate in the body with age, this may stimulate the inflammasome pathway, leading to chronic "smoldering" inflammation, called inflammaging, which sometimes acts as a trigger for tumor proliferation (18, 40). This is an "internal inflammation" without an infectious agent, in which the pro-tumor effects of inflammatory cells include, among other things, the secretion of mutagenic forms of oxygen and nitrogen, the provision of growth factors, and enzymes that promote cell

proliferation, mutagenesis, angiogenesis, and metastasis (15). Thus, inflammation can both induce cancer and, less commonly observed, cancer induces internal inflammation as a response to its action (21) (Fig. 3). Such smoldering inflammation is often enough to induce cancer, e.g. gastric inflammation due to *Helicobacter pylori* (CagA protein). Johannes A. Fibiger, a Danish Nobel laureate in 1913, was the first to experimentally induce gastric cancer in rats infected with nematodes, which metastasized and could be transplanted to other animals, although it is now believed that the cause of this pathology was chronic inflammatory response induced by parasite irritation. Nevertheless, it is currently accepted that chronic inflammation and infectious agents are responsible for 15-20% of cancers (29). The mentioned inflammatory response is also an example of the influence of the microenvironment on oncogenesis and its progression: TME (tumor microenvironment – tumor environment, e.g., oxygen levels, pH) and *vice versa* (3, 6, 16-18, 39). Mutation of the environment genes of the cell (landscape genes) promotes the creation of conditions for their uncontrolled proliferation (43, 45). This manifests itself in a specific inflammatory response that affects all stages of oncogenesis by acting not only on tumor cells but also on stromal cells and immune cells. Currently, it is believed that the main mediators between inflammation and cancer are the transcription factors NF-κB (nuclear factor κB) and STAT (signal transducers and activators of transcription) (2, 8, 10, 30, 31, 43).

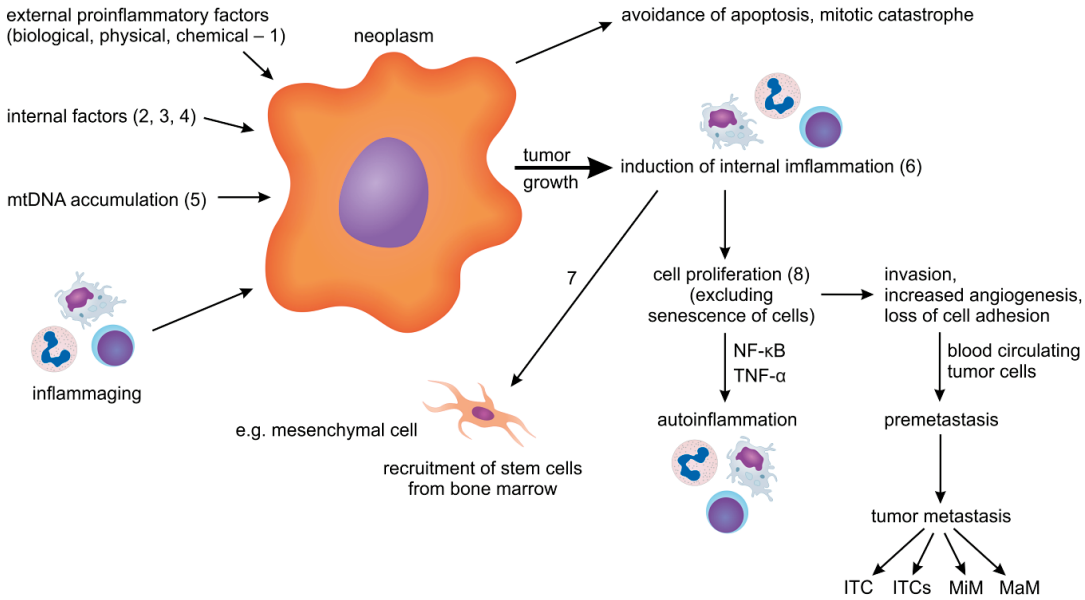

Fig. 3. Tumor induced by inflammation and sterile internal inflammation induced by the presence of cancer

Explanations: 1 – e.g., asbestos or erionite → lung inflammation → cancer (mesothelioma); cigarette smoke (73 carcinogens) → increased expression of *RAS*; 2 e.g. a – effects of obesity: macrophages stimulation; pro-inflammatory gene expression profile; disorders in adipokine secretion; numerous types of cancer, e.g., excess insulin as a compensatory mechanism for insulin resistance → MAPK (mitogen-activated protein kinase) stimulation → accelerated mitosis, disrupted control of the differentiation process of cells; b – nonspecific bowel inflammation → colon cancer; 3 – oncogenes *K-RAS*, *MYC*, *TP53* gene; 4 – inflammation due to DNA damage induced by damage-associated molecular patterns (DAMP); 5 – through inflammasome activation and release of pro-inflammatory cytokines, impaired autophagy; 6 – internal inflammation refers to the body's response to cancer or cancer-induced inflammation; 7 – e.g., prostate cancer recruits mesenchymal stem cells (MSC) through the chemoattractant CXCL16; 8 – mutagenesis, angiogenesis, metastatic cancer spread, ITC (isolated tumor cells), ITCs – scattered isolated tumor cells (focus up to 0.2 mm), MiM – micrometastases (0.2-2 mm), MaM – macrometastases (> 2 mm)

In cancer metastasis, mainly in cell extravasation, integrins regulated by ADAM (a disintegrin and metalloproteinases), proteins play a role, enabling cell movement by blocking integrins and binding cells to ECM components (41). Moreover, aging and chronic inflammation impair autophagy ("self-eating", a type II cell death), among other things by disturbing the expression of proteins that induce it (e.g. Bak-1), which leads to uncontrolled proliferation and induction of cancer. This response can be suppressed by stimulating autophagy, resulting in an increase in CD8+ T lymphocytes and a decrease in Treg (regulatory) lymphocytes, which favors the survival of cancer cells and correlates with antitumor immune activity (44). Excessive autophagy and mitotic catastrophe (caused by abnormal mitosis) are alternative forms of cell death, leading to non-apoptotic cell death. Defects in genes, such as BECN1 in these processes, can become oncogenic (27). Cancer cells can also consume apoptotic products, even digesting entire cells (entosis), but they do not participate in new proliferation (29). It has also been noted that autophagy pathways may be disorganized in cancer, as their cells then divide without initiating this process and without cannibalizing their organelles. However, there are situations where cancers using autophagy in extreme energy deficiency states enter a dormant and hibernation state for varying periods of time, which complicates cancer therapy (10).

In the microenvironment and in the surrounding ECM, cancer cells can communicate with non-cancerous cells using exosomes, which are multi-vesicular bodies, transferring DNA, RNA (including miRNA – microRNA), and proteins through fusion of their contents or ligand-receptor interactions, known as horizontal transfer (21, 30). Through this mechanism,

exosomes can, among other things, reprogram stromal cells, induce inflammatory processes, and help remodel the ECM and recruit cells from the bone marrow and immune system. All this promotes the formation of a pre-metastatic cancer niche (27). miRNAs are 18-25 nucleotides long RNA molecules that regulate mRNA expression (without coding proteins). In cancer, they can act as oncogenes (so-called oncomirs) or tumor suppressors (28). Overexpression of a single miRNA (e.g., mir-55) can, for example, induce cancer in mice (21). miRNA transfer through gene expression can also stimulate normal cells *in vitro* to form tumors (30).

Rapid tumor proliferation, followed by hypoxia and cell death, can induce inflammation. The inflammatory process also stimulates cancer metastasis through direct regulation of transcription factors such as TWIST/SLUG. Similarly, transcription factors like NF-κB and STAT3 can induce stem cells and pro-inflammatory chemokines, promoting angiogenesis and eventually tumor metastasis (29).

Although cancer cells acquire the ability to invade surrounding tissues and metastasize through mutations and epigenetic changes, the tumor microenvironment is a critical structure for them, as it provides signals that enhance these traits. Mutations, often somatic, can also occur in stromal cells, stimulating mitosis and increasing the survival of tumor cells. These abilities may be promoted or accelerated by chronic inflammation; e.g. inflammaging, and modulated by the tumor microenvironment, in which many immunosuppressive factors are active (34). In a tumor, T lymphocytes are inhibited by B cells, i.e. cells that enhance the inflammatory infiltration together with Treg lymphocytes (1). A T lymphocyte with a programmed cell death receptor 1 – PD-1 can interact with cancer cell molecules (PD-L1 and PD-L2 – ligands), inducing T cell death (45). The result is local immunosuppression. Antitumor responses are also inhibited by molecules such as CTLA-4 (cytotoxic lymphocyte-associated 4 – receptor 4, associated with Tc lymphocytes), which compete with the CD28 molecule, preventing costimulation of T lymphocytes through this pathway (1). This molecule inhibits Tc lymphocytes and enhances the control exerted by Ts lymphocytes (suppressor cells). This reaction occurs when the APC (antigen-presenting cell), containing CD80 (B7-1) and CD86 (B7-2) molecules on its surface, activates T lymphocytes (10). Immunosuppression in the microenvironment also involves immune cells such as Treg lymphocytes (mainly pTreg – peripheral Treg lymphocytes), Th17, N2 phenotype neutrophils, M2a, M2b, M2c, and M2d macrophages (influencing the development of tolerance to cancer – TAM – tumor-associated macrophages), MSC (mesenchymal stem cells), and MDSC (Gr, Mo, e – MDSC types) (1, 45).

Recently, much attention has been paid to the role of macrophages, which, for example, in breast cancer, may constitute 50% of the tumor mass and "switch" the canonical Wnt pathway to the non-canonical one by inhibiting the expression of canonical Wnt inhibitors such as Wnt-2, Wnt-5a, DKK-1, ROR2, and β-catenin (23). Thus, the inhibition of the canonical Wnt pathway in cancer cells by macrophages is not a result of their "corruption" by cancer cells to support tumor proliferation, but rather the result of their antitumor activity. Inhibitors of the canonical Wnt pathway are, in fact, ligands for the non-canonical Wnt pathway, and the side effect is an increase in invasiveness and the ability to metastasize (23). The presence of fibroblasts, in turn, leads to increased expression of EMT-related genes in them, a key element of tumor cell malignancy.

Pro-inflammatory M1 phenotype macrophages also infiltrate adipose tissue and surround necrotic adipocytes, forming structures resembling crowns and becoming activated by saturated fatty acids released by dying adipocytes (1). These macrophages, called MTM (adipose tissue macrophages), are very heterogeneous, and their function is not yet fully understood. For example, in the perivascular adipose tissue associated with atherosclerotic plaques, about 29% of macrophages are gathered, and after plaque rupture, they constitute 26% of all cells. They secrete MMP and eventually express CCR5, CXCR3, and TLR2, thus having repair, cytotoxic, and iron-using abilities during plaque rupture (1). Macrophages also produce pain-relieving endorphins (proenkephalin, dynorphin, and endomorphin), and under the influence of the cytokine IL-1β, they release endogenous opioids, as well as glucocorticoids of adrenal cortex origin (mediated through IL-6 and proopiomelanocortin), which can alleviate the symptoms of cancer (1).

The formation of pluripotent stem cells from mature (adult) cells suggests that aging could be a reversible process. However, replacing senescent cells in humans with new cells raises concerns, as the former are often essential for life, such as neurons that transmit information, without which we would not remember anything. Similarly, disabling senescence would prevent aging but significantly accelerate the appearance of cancers in early youth. Cancers acquire somatic mutations during life, as inherited mutations rarely induce them, even though humans carry about 4 million genetic variants, but these changes are prevalent in cancer cells, making their therapy much more difficult.

The current goal of therapy for eliminating cancer cells is to activate the immune system so that it can autonomously destroy the cancer within the microenvironment (1). Cancer cells evade apoptosis, anoikis, and aging, ensuring their immortality through telomerase reactivation, and their metastases may also be more homogeneous than the primary tumor and differ from it genetically (29, 42).

Cancer-induced inflammation was described in a previous study (15). Currently, inflammation propagated by cancer is presented as a trigger for its malignancy. For example, a chronic inflammatory state

induced by autoimmune diseases (such as Hashimoto's thyroiditis, Sjögren's syndrome in the salivary gland, *Helicobacter pylori* infection in the stomach) can generate extranodal marginal zone lymphoma (MZL), composed of mature B cells with CD20 expression and surface IgM (10).

Inflammatory cells and resident ECM (extracellular matrix) cells participate in many phenomena, such as acting as growth suppressors (proteases degrading adhesive molecules), producing factors that promote proliferation (EGF, proteases), influencing the resistance to death (macrophages inhibiting anoikis), invasion and metastasis (TGF-β → promotion of EMT, TNF and EGF → increased migration of tumor cells, macrophages → ECM remodeling), angiogenesis (VEGF → stimulation of the process), and evading immune destruction (TGF-β → recruitment of Treg cells – regulatory T cells, suppression of CD8+ T cells, M2 macrophages → promotion of angiogenesis, fibroblasts, and collagen deposition via cytokines) (10).

In conclusion, it can be stated that oncogenesis in old age occurs by means of: 1 – a direct pathway through the excessive production of mediators by senescent cells at subsequent stages of oncogenesis, or/and 2 – an indirect pathway via the aforementioned mediators involved in the process of inflammaging. Furthermore, in this reaction, immune system cells are recruited by pro-inflammatory substances secreted by senescent cells.

Aging cells undergo changes that modulate their constitutive functions, such as acquiring a "reluctance" to die and displaying new, induced properties (ambivalence toward tumor development). Thus, the aforementioned cells can act both as stimulators (promoters) and suppressors of tumor development. Suppression can occur through the elimination of tumor cells by the immune system when it recognizes tumor-specific antigens (TSA) and tumor-associated antigens (TAA) as "foreign" (29).

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