



## Inflammation in the molecular pathogenesis of cancer and atherosclerosis

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### ABSTRACT

Chronic inflammation, atherosclerosis and cancer are leading causes of death in industrialized society. Epidemiological studies have shown that chronic inflammation predisposes individuals to certain cancers, while anti-inflammatory and anti-oxidant agents may protect against cancer development and metastasis. Inflammation supports the different phases of cancer development through the inflammatory molecules produced by infiltrating immune cells, resident stromal cells and even cancer cells. Although atherosclerosis has been considered to be multi-factorial disease, in which genetic and environmental factors have been implicated, inflammation also significantly contributes to plaque formation and progression, and to stenosis of atherosclerotic lesions. Major nuclear transcription factors and molecular mediators of inflammation that induce altered cell expression of adhesion molecules, proteases, and growth factors are common factors in the microenvironment leading to disease development and progression of both atherosclerosis and cancer. Important pathogenic pathways on atherosclerosis and cancer follow endothelial cell dysfunction and the activation of the hemostatic system and angiogenesis via inflammation-dependent mechanisms represent important features of this dysfunction. Therefore, novel target-oriented therapies affecting altered mechanisms of inflammation, angiogenesis and tissue proliferation may similarly inhibit atherosclerosis and cancer. Main treatment strategies include reducing oxidative stress; inhibiting chemokine, cytokine, and growth factor cell signal transmit; down-regulating excess matrix digestion; inactivating nuclear factor-kappa B signal pathway, and interfering with cell cycle regulation.

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### La inflamación en la patogenia molecular del cáncer y la arteriosclerosis

#### RESUMEN

Las enfermedades inflamatorias crónicas, la arteriosclerosis y el cáncer están entre las primeras causas de muerte en la sociedad industrializada. Estudios epidemiológicos han demostrado que las enfermedades inflamatorias crónicas predisponen a la aparición de ciertos cánceres, mientras que algunos agentes antiinflamatorios y antioxidantes protegen frente al desarrollo del cáncer y sus metástasis. La inflamación facilita diferentes fases del desarrollo del cáncer, a través de moléculas producidas por células del sistema inmunitario infiltrantes de los tumores, por células del estroma tumoral e, incluso, por las propias células tumorales. Aunque la arteriosclerosis se ha considerado como una enfermedad de etiología multifactorial, en la que están implicados factores genéticos y ambientales, la inflamación también contribuye a la formación y el desarrollo de la placa e, incluso, la estenosis vascular en las propias lesiones arterioscleróticas. Los principales factores nucleares de transcripción y mediadores moleculares de la inflamación que alteran la expresión de moléculas de adhesión, proteasas y factores de crecimiento, son factores habituales del microambiente que conduce al desarrollo de la arteriosclerosis y el cáncer. De hecho, sus rutas patogénicas más importantes dependen de alteraciones del endotelio vascular y la activación del sistema hemostático y la angiogenia a través de mecanismos inflamatorios. Por consiguiente, los nuevos tratamientos orientados a dianas moleculares específicas, que afectan a mecanismos alterados de inflamación, angiogenia y proliferación tisular, podrían inhibir igualmente la arteriosclerosis y el cáncer. Entre las principales estrategias a considerar están la disminución del estrés oxidativo, la inhibición de algunas quimiocinas, citocinas y transductores intracelulares de factores de crecimiento, la regulación negativa de la digestión de matriz extracelular, la inactivación del factor nuclear kappa B, y la interferencia de reguladores del ciclo celular.

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#### Palabras clave:

Inflamación

Cáncer

Metástasis

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Citocinas

Angiogenia

Estrés oxidativo

### Chronic inflammation: tissue recovery versus disease progression

Chronic inflammation has been implicated in the pathogenesis of autoimmunity, atherosclerosis, and cancer. Inflammation is the

initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and strengthens the inflammatory response, involving the local microvascular system, the immune system, the connective tissue and parenchymal cells within the microenvironment of injured tissue. The recovery of the acute phase of inflamed

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tissue is involved with the following processes: First, inflammatory factors with proangiogenic effects—for instance vascular endothelial growth factor (VEGF)—are upregulated in the inflamed tissues in order to stimulate new blood vessel development, which in turn increases blood infusion to the damaged tissue. This process is crucial for the supply of oxygen, nutrients and leukocytes to the lesion area that needs tissue repair and healing. Second, part of the protective mechanisms of the body against antigens and pathogens in the inflamed tissue is the release of reactive oxygen species (ROS) in order to harm pathogens. Third, replacement of necrotic and apoptotic tissue cells through the activation of cell proliferation and regeneration via massive release of growth factors that enhance signal transduction processes. These three processes are mediated by activated neutrophils, monocytes and endothelial cells, which are the major cell populations supporting the proinflammatory response at inflamed tissues.

Prolonged inflammation, also known as chronic inflammation, leads to simultaneous destruction and healing of the affected tissue and to the progressive shift in the type of cells that are present at inflammatory sites. Therefore, although inflammation is central to our fight against pathogens, if it is not ordered and timely, the resulting chronic inflammation can contribute to diseases such as heart attacks, arthritis and Alzheimer's disease. Chronic inflammation has also become a recognized risk factor for carcinogenesis and can be caused by sustained infection, autoimmune disorders and other pathologies. It results in the infiltration of inflammatory cells at specific sites in the body, including macrophages, T and B cells, natural killer cells, neutrophils and other granulocytes. T cells and macrophages are the predominant inflammatory cells and they excrete large amounts of inflammatory cytokines, proangiogenic factors and reactive oxygen species (ROS) into the microenvironment.

Angiogenesis, secretion of ROS and growth factor-dependent tissue regeneration are the outcome of the activation of both leukocytes and endothelial cells, and have beneficial effects during the acute phase of inflammation. However, during chronic inflammation, the same processes can become very harmful. Increased blood supply, which brings oxygen and nutrients in the presence of a lot of growth factors, can trigger uncontrolled cell division; massive ROS release can damage DNA and subsequently can give rise to mutated transformed cells; and the stimulation of proliferative signal transduction pathways via massive release of growth factors can support not only regeneration and healing of normal tissue, but also transformed cell development.

Angiogenesis in the chronic inflamed tissue is a consequence of at least two different mechanisms: The first is proangiogenic factor secretion by pathogen-activated monocytes and neutrophils. These two cell types, upon triggering with endotoxins or cytokines such as IL-1 $\beta$ , secrete proinflammatory and proangiogenic factors such as VEGF. The second is the release of proangiogenic factors by endothelial cells at the inflamed tissue. Under conditions of injury, infection, or chronic inflammation, endothelium is exposed to agonists such as endotoxin, thrombin, and heparan sulfate, and in response, it expresses both plasminogen activator inhibitor 1 (PAI-1), which promotes coagulation; and E-selectin, interleukins (IL-1 and IL-6) and chemokines (IL-8), which further stimulate inflammation. This microvascular activation was first described in endothelial cells exposed to endotoxins, such as LPS; and cytokines, such as IL-1, TNF $\alpha$  or IL-6. Endotoxins activate endothelial cells by stimulating toll-like receptor 4 (TLR4), and IL-1 stimulates one or more of several IL-1 receptors (IL-1R). In turn, TLR4 and IL-1R invoke well-known intracellular signaling pathways that activate NF- $\kappa$ B, a transcriptional regulator that orchestrates expression of proinflammatory and proangiogenic genes in endothelial cells. The

activation of TLR4 and IL-1 receptors on immune and endothelial cells leads to the secretion of proangiogenic cytokines, such as VEGFA and VEGFB, in turn acting on VEGFR1-, VEGFR2- and VEGFR3-expressing cells to promote new blood vessel formation at the inflamed tissues.

The fibrinolytic system<sup>1</sup>—represented by the cellular receptor of urokinase uPAR, urokinase (uPA) and its specific inhibitor PAI-1—, can also promote cancer progression and metastasis.<sup>2</sup> Inflammatory cytokines not only upregulate PAI-1 but uPA also activates NF $\kappa$ B via IKK. There is a link between the uPAR/uPA/PAI-1 system and inflammation-dependent tumor initiation and progression. These inflammatory matrix proteins are responsible for the altered composition of the inflammatory matrix containing large amounts of PAI-1. Urokinase is also a plasminogen activator that transforms the zymogen plasminogen into the active protease plasmin. Plasmin in turn can degrade the extracellular matrix and activate other proteins (i.e. pro-metalloproteases and pro-growth factors). In human tumors, the excessive expression of uPA and uPAR is a marker for an unfavorable clinical outcome. Moreover, in human tumor xenografts, uPAR blockade inhibits cancer growth and metastasis. The uPAR/uPA/PAI-1 system is also involved in VEGF-induced angiogenesis, thereby further contributing to tumor progression. Finally, a variety of uPAR interactors such as integrins, growth-factor receptors, G-protein coupled receptors, members of the LDL receptor family and vitronectin appear to induce cancer cell phenotype. Altogether, fueled by inflammation the uPAR/uPA/PAI-1 system in turn influences tumor initiation and progression via several pathways that can also become targets for inhibition.

### Inflammation in cancer development and metastasis

There is a poorly understood, but longstanding, observation and epidemiologic link between inflammation and cancer. It was in 1863 that Rudolf Virchow reported for first time leucocytes in neoplastic tissues and made a connection between inflammation and cancer. He suggested that the “lymphoreticular infiltrate” reflected the origin of cancer at sites of chronic inflammation. Recent estimates suggest that about 20% of all human cancers are caused by chronic infection or chronic inflammatory states.<sup>3,4</sup> Prominent examples for this phenomenon are the strong associations between chronic gastritis, hepatitis, prostatitis or colitis and increased risk of primary carcinoma in the corresponding organ. Almost 30% of people who suffer from inflammatory bowel disease will develop colorectal cancer, and around 18% of people who suffer from inflammation of the prostate will develop prostate cancer.<sup>5</sup> Similar numbers exist for the development of liver cancer from hepatitis.<sup>6</sup> Therefore, the development of cancer cells from chronic inflamed tissues is a major problem in the world.

It has been suggested that chronic inflammation supports the different phases of tumor development, i.e. initiation, promotion and progression. Inflammatory molecules that are produced by resident tissue cells and infiltrating defense cells, including macrophages, lymphocytes, natural killer cells, neutrophils, dendritic cells and eosinophils, in part provide this support. For instances, *Helicobacter pylori*-induced gastritis is associated with gastric carcinoma and gastric B-cell lymphoma.<sup>7</sup> Chemokines induced by *H. pylori* attract B-cells to the mucosa where they become targets for the carcinogenic process that can occur during the chronic inflammatory process. However, biochemical processes in chronic inflamed tissues that are responsible for the development of cancer are still unclear. The transformation of chronic inflamed tissue into cancerous tissue is a process comprising of at least three stages. The first stage is the

development of chronic inflamed tissue from an acute inflamed one. The second is the “maturation” of chronic inflammation, which includes build-up of new blood vessels, destruction of the old tissue and assembly of a new tissue. The third stage is the development of cancer cells in the chronic inflamed tissue.

Two major processes should take place in order for a tumor to develop in the inflamed tissue: 1) transformation of the normal cells into the cancer ones and 2) intensive blood supply of the transformed cells by neo-vessels formation (angiogenesis). Both of these processes are not independent, but influence each other: cancer cells release large amount of the proangiogenic factors, which intensify angiogenesis, while new blood vessels formation brings ROS into the microenvironment that damages DNA and increases the number of the mutated transformed cells. This situation can considerably decrease the effectiveness of the attempts to inhibit the harmful processes separately and challenges searching of ways of simultaneously suppressing both of them.<sup>8</sup>

There are several pathways through which inflammatory mediators or products of inflammatory activated cells could affect tumor initiation and progression.<sup>9</sup> On the one hand, cytokines produced by activated innate immune cells can stimulate abnormal epithelial cell growth, while soluble mediators produced by altered epithelial cells can further recruit and activate inflammatory and stromal cells, and these processes might even promote immune-suppression at the carcinogenic microenvironment. Interestingly, activation of the humoral immune system during the inflammatory process could be linked to inflammation-mediated tumor progression. On the other hand, during tumor initiation—i.e., when genomic alterations and modifications in gene expression transform a normal cell into a pre-malignant cell—, activation of epithelial cells by inflammatory cytokines such as TNF can result in NF $\kappa$ B-dependent upregulation of an array of genes among those also inhibitors of apoptosis genes that in concert with other molecules inhibit apoptosis. This results in a higher resistance of inflammatory activated epithelial cells to become apoptotic, and in turn better survival of cells with a “malignant” mutation that under non-inflammatory conditions would induce apoptosis of such cells.

Moreover, in some human and mouse cancers, the transformed cells themselves can also contribute to the overall levels of soluble pro-inflammatory cytokines. For example, TNF $\alpha$  produced by keratinocytes is a tumor promoter in experimental skin cancer, where it produces a cascade of cytokines and of enzymes that degrade the extracellular matrix during the early stages of tumor promotion. Human keratinocytes also produce TNF $\alpha$  in response to ultraviolet irradiation. Moreover, activation of NF $\kappa$ B occurs in many malignant cells as a result of genetic mutation rather than in response to signals from surrounding cells. NF $\kappa$ B pathway may not affect initiation but may have dual actions in tumor promotion:<sup>10</sup> first by preventing the death of cells with malignant potential, and second by stimulating the production of pro-inflammatory cytokines by tumor-associated stromal cells. So, pro-inflammatory cytokines contribute to tumor promotion not only by signaling from tumor-associated inflammatory cells to precancerous cells, but also through production in the precancerous cells themselves, especially at early stages. Altogether, a whole array of molecular mechanisms depending on inflammation, which can influence tumor initiation and progression.

Finally, cancer metastasis development, as a consequence of the systemic dissemination of cancer cells, can also be promoted by major inflammatory cytokines inducing cancer cell adhesion, migration and invasion.<sup>11</sup> In the liver—the second most commonly involved organ by cancer metastasis after the lymph nodes—, we have reported that proinflammatory cytokine release from tumor-activated sinusoidal cells is an early, tumor-specific

inflammatory response to liver-invading cancer cells, that influences metastasis occurrence.<sup>12</sup> In addition, factors that either attenuate tumor-induced host proinflammatory response or adhesion receptors for cancer cells may have a therapeutic potential in the prevention of liver metastasis. In addition, transdifferentiation of perisinusoidal hepatic stellate cells by tumor-derived factors also results in the generation of tumor-associated myofibroblasts whose release of inflammatory response gene products, such as growth factors, extracellular matrix proteins and a proteolytic armory degrading and remodeling matrix, further promote angiogenesis and tumor growth.<sup>12</sup>

### Inflammation in atherosclerosis

Atherosclerosis has been viewed to reflect the deposition of lipids within the vessel wall of medium-sized and large arteries. Despite the fact that the association between LDL cholesterol and atherosclerosis has been evident for at least three decades, our understanding of exactly how LDL precipitates atherosclerosis is still unclear. Now this concept has changed and it is assumed that a complex endothelial dysfunction induced by elevated and modified low-density lipoproteins, free radicals, infectious microorganisms, shear stress, hypertension, toxins after smoking, or combinations of these and other factors, can lead to a compensatory inflammatory response.<sup>13</sup> Endothelial dysfunction is characterized by decreased nitric oxide synthesis, local oxidation of circulating lipoproteins and their entry into the vessel wall. Intracellular ROS, similarly induced by the multiple atherosclerosis risk factors, lead to enhanced oxidative stress in vascular cells and further activate intracellular signaling molecules involved in gene expression. Upregulation of cell adhesion molecules facilitates adherence of leukocytes to the dysfunctional endothelium and their subsequent transmigration into the vessel wall. The evolving inflammatory reaction is instrumental in the initiation of atherosclerotic plaques and their destabilization, and, therefore, inflammation is considered to play an important role in the progression of atherosclerosis and artery plaque destabilization, converting a chronic process into an acute disorder with ensuing thrombo-embolism.<sup>14</sup>

During atherosclerosis, T cells and macrophages infiltrate the vessel wall triggered by endothelial dysfunction, and locally interact in a synergistic manner. Auto-reactive T cells recognize oxLDL, heat shock proteins and share microbial antigens by molecular mimicry and release proinflammatory cytokines. Macrophages on stimulation by T-cell-derived cytokines and transformation into foam cells after uptake of oxLDL, also secrete matrix metalloproteases predisposing the plaques to subsequent rupture. Plaque-associated macrophages, moreover, are an important cellular source of tissue factor. Finally, on plaque rupture, tissue factor-rich plaque material gets in contact with the circulation and activates the extrinsic coagulation pathway.

The fact that proinflammatory cytokines are instrumental in the progression of atherosclerosis, as revealed by numerous animal studies and suggested by their expression in atherosclerotic human plaques, opens the therapeutic prospect of targeting cytokine expression and cytokine-signaling proteins. In experimental settings, blockade of IFN- $\gamma$  and TNF $\alpha$  ameliorates atherosclerosis development. Pentoxifylline—a TNF antagonist—inhibits plaque formation in apoE $^{-/-}$  mice by shifting T cells toward T-helper-2 differentiation, characterized by increased production of immune suppressant cytokine IL-10. As another approach, inhibitors of matrix metalloprotease activity and suppressors of cytokine signaling proteins can also ameliorate cytokine-induced chronic inflammation in the vessel wall. Current therapeutics effective in preventing atherosclerosis and stroke

such as statins, acetylsalicylic acid and renin-angiotensin system inhibitors may also exert part of their effects by modulating inflammatory responses in the vessel wall.

### Inflammation in the pathogenic intersection between atherosclerosis and cancer

Atherosclerosis and cancer are the most important source of morbidity and mortality in the developed world. Both chronic diseases appear to be multi-staged in their progression, with genetic, nutritional, psycho-social, environmental and viral factors influencing their appearances. However, major molecular inflammatory pathways and their nuclear transcription factors, such as NF $\kappa$ B, have a significant role in the pathogenesis and progression of both atherosclerosis and cancer. For example, alteration of cytokine-dependent cell adhesion molecules, such as integrins and cadherins, have been linked to plaque formation and thrombosis, as well as to cancer invasion and metastasis. Altered expression of proteases associated with thrombolysis has also been implicated in atherosclerotic plaque expansion and hemorrhage, and in the pathogenic process of cancer invasion and metastasis. Ligand-growth factor receptor interactions (tyrosine kinases) have been associated with early atherosclerotic lesions, as well as with cancer development and spread. Moreover, pro-angiogenic inflammatory factors have recently been linked to plaque expansion and restenosis of atherosclerotic lesions as well as cancer cell spread from primary tumors and metastatic tumor growth.<sup>15–17</sup> On this basis, as we move forward in our understanding of these diseases, efforts are increasingly focused on the inflammatory mechanisms underlying disease activation, that precipitate major clinical manifestations of both atherosclerosis—heart attack and stroke—, and cancer,—primary tumor invasion and distant metastasis—. Moreover, novel target-oriented therapies affecting altered mechanisms of inflammation, angiogenesis, matrix remodeling and tissue proliferation have similarly inhibited atherosclerosis and cancer. Main treatment strategies in clinical development include reducing oxidative stress; inhibiting chemokine, cytokine, and growth factor cell signal transmit; down-regulating excess matrix digestion; inactivating nuclear factor-kappa B signal pathway, and interfering cell cycle regulation.

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