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ОСОБЕННОСТИ ПАТОГЕНЕТИЧЕСКИХ МЕХАНИЗМОВ РАЗВИТИЯ АТЕРОСКЛЕРОЗА И СТАРЕНИЯ

© Аршед Ахмад Кучай[1, 6], Александр Николаевич Липин[1, 2], Кирилл Ленарович Козлов[2, 3], Никита Николаевич Груздев[1], Гузель Шамиловна Кучай[5], Александр Александрович Шугаров[4]

[1] Центр спасения конечностей, СПб ГБУЗ Городская больница № 14. 198099, г. Санкт-Петербург, ул. Косинова, д. 19
[2] Военно-медицинская академия им. С.М. Кирова. 194044, г. Санкт-Петербург, ул. Академика Лебедева, д. 6
[3] Институт биорегуляции и геронтологии. 197110, г. Санкт-Петербург, пр. Динамо, д. 3
[4] Курский государственный медицинский университет. 305000, г. Курск, ул. Карла Маркса, д. 3
[5] ИПКА Лабораториз. 198095, г. Санкт-Петербург, ул. Ивана Черных, д. 2
[6] Многопрофильная медицинская клиника «Диона». 194354, г. Санкт-Петербург, пр. Луначарского, д. 21/1

Контактная информация: Аршед Ахмад Кучай — врач сердечно-сосудистый хирург, клинический исследователь. E-mail: drarshedcvs@gmail.com ORCID: https://orcid.org/0000-0002-7974-9369 SPIN: 5455-9033

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РЕЗЮМЕ. Атеросклероз относят к болезням старения, так как повышение возраста является независимым фактором риска его развития. Данный процесс связан с преждевременным биологическим старением, поскольку в атеросклеротических бляшках выявляются признаки снижения пролиферации клеток, апоптоза, повышенного повреждения ДНК, эпигенетических модификаций, укорочения и дисфункции теломер. Атеросклероз является хроническим воспалительным заболеванием внутренней стенки крупных и средних артерий. Это основная причина развития сердечно-сосудистых и цереброваскулярных заболеваний. Патоморфологически атеросклероз представляет собой хроническое воспаление артерий, вторичное по отношению к длительному воздействию окислительного стресса, в процессах которого участвуют многочисленные типы клеток и медиаторы. Окисленные липиды, образующиеся из липопротеидов низкой плотности, способствуют развитию и прогрессированию атеросклеротических бляшек за счет выработки воспалительных цитокинов. Диета и пищевые привычки являются основными факторами, способствующими развитию и прогрессированию атеросклеротических заболеваний. Генетика и эпигенетика также оказывают значимое влияние на развитие и прогрессирование атеросклероза. В перспективе терапевтические подходы могут быть направлены на патогенетические медиаторы атеросклероза на молекулярном уровне.

КЛЮЧЕВЫЕ СЛОВА: атеросклероз, эндотелиальная дисфункция, микробиота, генетика, воспаление, окислительный стресс, старение

PECULIARITIES OF PATHOGENIC MECHANISMS OF ATHEROSCLEROSIS AND AGING

© Arshed A. Kuchay[1, 6], Alexander N. Lipin[1, 2], Kirill L. Kozlov[2, 3], Nikita N. Gruzdev[1], Guzel Sh. Kuchay[5], Alexander A. Shugarov[4]

[1] Limb Salvage Center, City Hospital № 14. 19 Kosinov str., Saint Petersburg 198099 Russian Federation
[2] Military Medical Academy named after S.M. Kirov. 6 Academician Lebedev str., Saint Petersburg 194044 Russian Federation
[3] Institute of Bioregulation and Gerontology, 3 Dinamo Ave., Saint Petersburg 197110 Russian Federation
[4] Kursk State Medical University, 3 Karla Marksa str., Kursk 305000 Russian Federation
[5] IPCA Laboratories, 2 Ivana Chernikh str., Saint Petersburg 198095 Russian Federation
[6] Multidisciplinary Medical Clinic “Diona”. 21/1 Lunacharsky ave., Saint Petersburg 194354 Russian Federation

Contact information: Arshed A. Kuchay — MD, MS. Cardiovascular surgeon, clinical researcher. E-mail: drarshedcvs@gmail.com
ORCID: https://orcid.org/0000-0002-7974-9369 SPIN: 5455-9033

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ABSTRACT. Atherosclerosis is classed as a disorder of aging, such that increasing age is an independent risk factor for the development of atherosclerosis. Atherosclerosis is also associated with premature biological aging, as atherosclerotic plaques show's evidence of reduced cell proliferation, irreversible growth and apoptosis, elevated deoxyribonucleic acid (DNA) damage, epigenetic modifications, and telomere shortening and dysfunction. Atherosclerosis is also a chronic inflammatory disease of the inner wall of large and medium-sized arteries. It is a disease of multiple causes which regarded as the primary underlying cause of heart diseases and cerebrovascular disorders. Pathologically, atherosclerosis is a chronic arterial inflammation secondary to prolonged exposure to oxidative stressors and involves multiple cell types and cellular mediators. Oxidized lipids derived from low-density lipoprotein contribute to multiple stages of atherosclerotic plaque development and progression through production of inflammatory cytokines. Diet and dietary habits are the major driving forces for development and modification of atherosclerotic diseases. Genetics and epigenetics have a significant influence on development and progression of atherosclerosis. Future therapeutic options may target the pathogenic mediators of atherosclerosis at multiple molecular levels.

KEYWORDS: atherosclerosis, endothelial dysfunction, microbiota, genetics, inflammation, oxidative stress, aging

INTRODUCTION

The term "atherosclerosis" has been coined by the German physician Felix J. Marchand (1846–1928) from the Greek words "athere" meaning gruel and "scleros" meaning hard [1]. Atherosclerosis and the subsequent cardiovascular complications, such as myocardial infarction, stroke, and ischemic heart failure, is a major cause of death in the Western world. The risk factors of atherosclerosis are well known, including hypertension, diabetes, serum total and low-density lipoprotein (LDL) cholesterol, and smoking. Atherosclerosis is defined as a chronic inflammatory disease of the inner wall of large- and medium-sized arteries [2]. From pathological perspectives, atherosclerosis has been defined as a chronic arterial inflammation secondary to prolonged exposure to oxidative stressors and involves multiple cell types and cellular mediators [3]. Arteries commonly affected include aorta, carotid arteries, coronary arteries and arteries of the lower extremities [4]. Atherosclerosis has been identified as a threat to mankind since 3000 B.C to 400 A.D following extensive investigation of mummies of the old world [4]. According to the review of literature, atherosclerosis was appeared to be an ancient disease process cross different races, as it was also found in mummies from China and North America [4]. Therefore, one may conclude that even though the terminology of atherosclerosis is recent but the disease itself is ancient [5–9]. This condition may lead to multiple vascular complications such as heart attack, coronary heart disease (CHD), stroke, peripheral arterial disease or even death [10, 11]. Increasing evidence indicates that aging is also an important risk factor for atherosclerosis and persists as an independent contributor when all other known factors are controlled. Premature or accelerated vascular aging can be promoted by cardiovascular risk factors [36, 37]. Despite variation in the underlying risk factors between countries, the first leading cause of death worldwide comes CHD. Based on the reports of the world health organization (WHO), 17.7 million people died in 2015 due to cardiovascular diseases (CVDs), which comprises about 31% of the worldwide deaths, of which; 80% are due to CHD and stroke [12]. The increasing burden of CVDs worldwide is attributed to the increasing incidence of atherosclerotic diseases [13].

PATHOGENESIS OF ATHEROSCLEROSIS

The basic mechanisms involved in pathogenesis of atherosclerosis (Fig. 1) are determined by

multiple factors of which inflammation, oxidation and genetic predisposition are the most important [14–17]. In the late 1970s, the "response to injury" theory was introduced by Russell Ross explained the pathogenesis of atherosclerosis as endothelial cell injury leading to endothelial dysfunction, followed by macrophage cell infiltration and smooth muscle cell (SMC) impaired function. In the last two decades, researches showed that atherosclerosis is a chronic inflammatory condition, which is the opposite of what previously thought on atherosclerosis to be a degenerative disease as a result of aging process. Because of the abundant researches in the field of atherosclerosis in recent years, understanding of pathophysiology of atherosclerosis made more and more accessible at lipoprotein metabolic level as well as at inflammatory molecular level [15, 16]. Recent understanding of pathogenesis of atherosclerosis (Fig. 2) explained it as

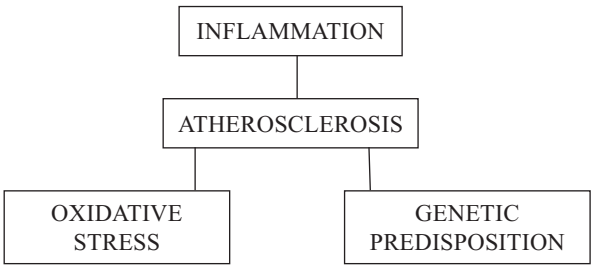


Рис. 1. Основные механизмы, участвующие в патогенезе атеросклероза

Fig. 1. Basic mechanism involved in pathogenesis of atherosclerosis

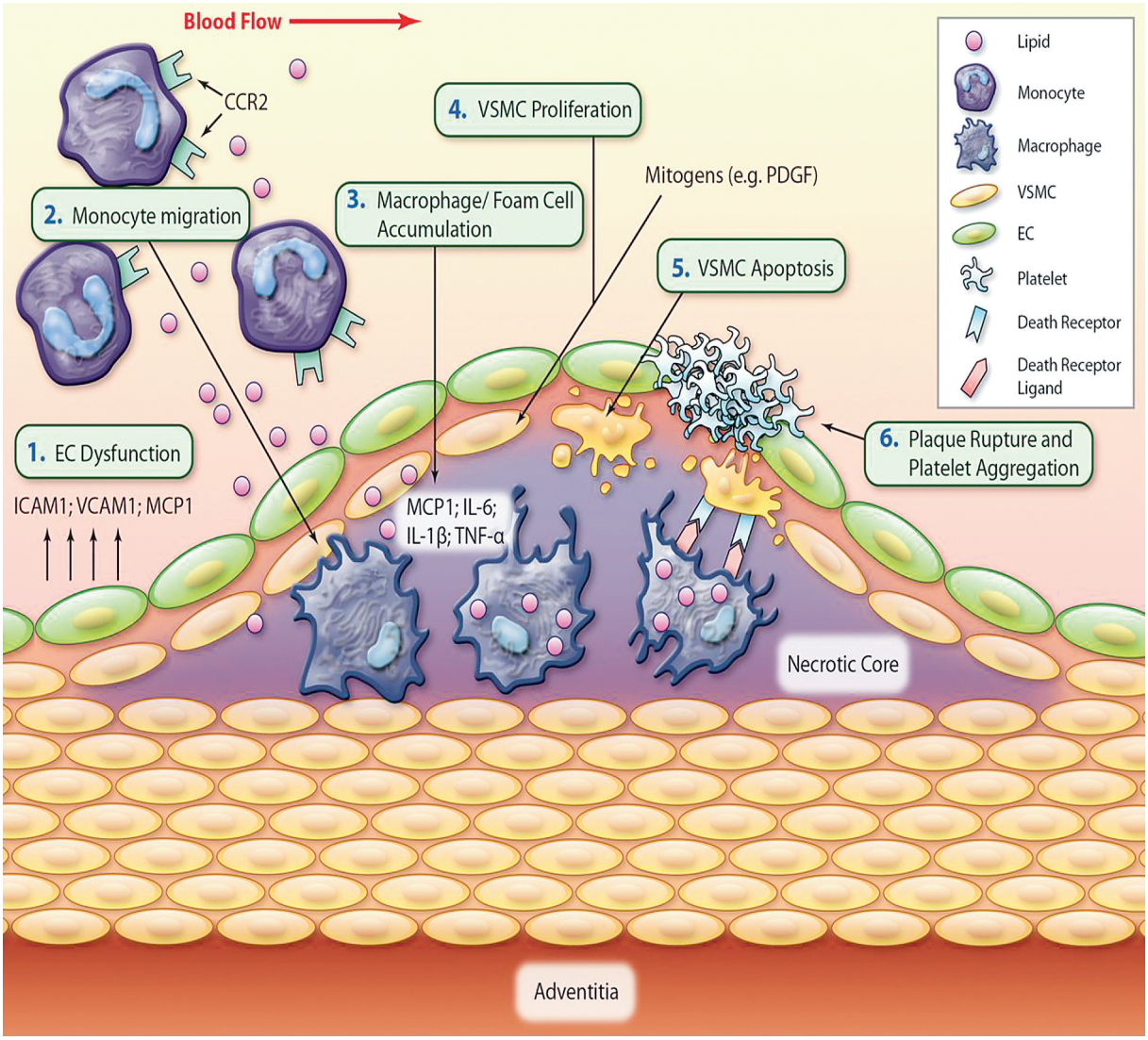
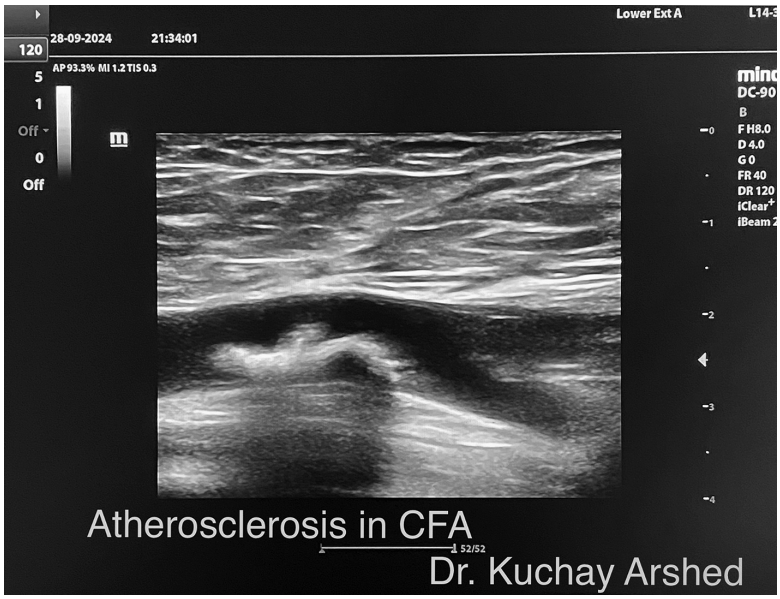


Рис. 2. Схема атерогенеза и формирования нестабильной атеросклеротической бляшки

Fig. 2. Schematic of atherogenesis and an unstable atherosclerotic plaque



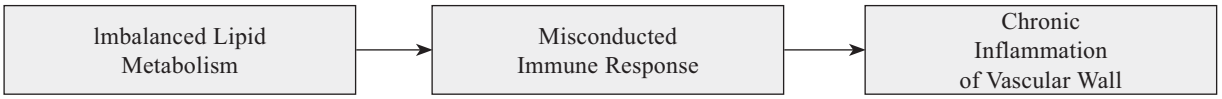


Рис. 3. Основные процессы патогенеза, участвующие в развитии атеросклероза

Fig. 3. Main pathogenesis process involved in atherosclerosis

imbalanced lipid metabolism integrated with misconducted immune response leading to chronic inflammatory process in the blood vessel wall.

The mature atherosclerotic plaque comprises an accumulation of vascular smooth muscle cells (VSMCs) and their secreted products (collagen and elastin), inflammatory cells (macrophages, T lymphocytes, dendritic cells, and mast cells), and both intracellular and extracellular lipid and debris (Fig. 2, 3). The latter is often concentrated in a necrotic core surrounded by a fibrous cap composed predominately of VSMCs. The stability of the atherosclerotic plaque depends on the thickness of the fibrous cap and the degree of cap inflammation. Plaque rupture is increased by cap thinning, promoted by death of VSMCs and breakdown of collagen and extracellular matrix (ECM), which may subsequently lead to myocardial infarction or stroke. However, plaque rupture is frequently subclinical, as VSMCs repair the rupture and reorganize associated thrombus. Indeed, complicated plaques frequently show evidence of multiple ruptures and repair, ultimately resulting in luminal narrowing. Successful plaque repair requires VSMCs to proliferate and synthesize matrix, both properties that are altered by cellular senescence. Indeed, cellular senescence can alter many of the proatherogenic events seen in Figure 2, 3.

The earliest histopathological lesion of atherosclerosis is the development of fatty streaks in the vascular wall at predilection points such as arterial branching sites where the laminar blood flow is disturbed [18]. A complex multiple processes of inflammation and oxidation are implicated in the development of fatty streaks in which LDL-c metabolism specifically appeared as a key factor. The biomechanical forces at arterial branching areas found to be the triggering factor of endothelial dysfunction creating barrier impairment (endothelial misalignment), proinflammatory process and prothrombotic function [17, 20, 25]. Once endothelial dysfunction induced, fatty streaks develop due to exposure of matrix proteoglycans and loss of confluent elastic layer of blood vessel lumen which commonly found at arterial bifurcation sites [27]. Recent updates indicate the role of hypercholesterolemia as a direct cause of endothelial dysfunction by changing the endothelial permeability hence allowing the migration of LDL-c into the vascular wall.

Atherosclerosis initiates upon endothelial dysfunction accompanied by low-density lipoprotein (LDL) retention and its modification in the intima. Modified LDLs, together with additional atherogenic factors, promote the activation of endothelial cells (ECs), leading to monocyte recruitment within the intima. Modified LDLs are avidly captured by differentiated monocytes

and VSMC, which promote foam cell formation [27]. In addition, several inflammatory signaling pathways are activated, allowing the fatty streak formation, which represents the first sign of atherosclerosis and is characterized by a substantial accumulation of lipids both within the cells (macrophages and VSMC) and the extracellular media.

Disruption of the mechanisms involved in vascular homeostasis regulation leads to endothelial dysfunction. Briefly, when ECs lose their ability to maintain homeostasis, vessel walls are predisposed to vasoconstriction, lipid infiltration, leukocyte adhesion, platelet activation, and oxidative stress, among other things [28]. Together, these induce an inflammatory response that is considered the first step of atheromatous plaque formation: the fatty streak. In addition, endothelial dysfunction also plays a remarkable role in subsequent steps of atherosclerosis by participating in plaque development and in its rupture in the last steps of atherosclerosis. Therefore, an increased endothelial dysfunction is considered an early indicator of atherogenesis.

Vessel aging, even in the absence of atherosclerosis, leads to intimal and medial thickening (vascular remodeling) as well as gradual loss of arterial elasticity, resulting in vascular stiffness [34, 38]. Aged vessels show a number of characteristic pathological processes, many of which are also seen in atherosclerosis [39, 40]. For example, aged vessels show reduced medial VSMC number, increased collagen deposition, and fracture of the elastin lamellae, which may lead to vessel dilation and increased lumen size [41]. Increased collagen and decreased elastin content, promoted at least in part by age-associated increases in glycated proteins, matrix metalloproteinase enzyme activity, and trophic stimuli such as angiotensin II signaling, impair vessel elasticity and hence promote vascular stiffness [42] and subsequently hypertension [43]. Hypertension can further stimulate collagen production with increased vessel stiffness and EC dysfunction. In addition to alterations in matrix and cell composition, aged vessels show elevated expression of a number of proinflammatory molecules [44] and increased uptake of plasma lipoproteins [45]. In part, these effects may be due to increased expression of leukocyte adhesion molecules on ECs in aged vessels, which trigger the familiar processes of monocyte migration followed by increased uptake of atherogenic lipoproteins with subsequent inflammation, key events that ultimately promote atherosclerosis (Fig. 2). Aged ECs and VSMCs also show increased secretion of proinflammatory cytokines (see below), resulting in persistent vascular inflammation. Thus, the effects of atherosclerosis are superimposed on normal aging of the underlying vessel.

A number of genetic diseases associated with premature aging also show the typical pathology of vascular aging and are prone to cardiovascular complications including atherosclerosis. For example, Hutchinson Gilford progeria syndrome (HGPS) is a rare, fatal, and progressive premature aging condition due to defects in lamin A that forms nuclear lamina. HGPS vessels from young patients reveal accumulated collagen, fractured elastin lamellae, and a thickened intima, with some vessels showing advanced atherosclerotic lesions containing chronic inflammation, calcification, and VSMC loss [46]. Mouse models of HGPS recapitulate the vascular pathology in patients and also show increased vascular stiffness and hypertension, supporting the concept of premature vascular aging [47]. Another example is Werner syndrome (WS), a loss-of-function mutation in the WS adenosine triphosphate (ATP)-dependent helicase (WRN), which shows a similar pathology and accelerated atherosclerosis. WS knock-out mice also show insulin resistance and increased blood glucose, increased circulating cholesterol, and enhanced lipid deposition [48], indicating that intrinsic vessel aging is superimposed on metabolic effects to promote vascular disease. HGPS and WS serve as important examples to understand any causal relationship between vascular disease and aging.

Because atherosclerosis compounds the pathological changes associated with normal vascular aging and vascular aging promotes atherosclerosis, this brings up the “chicken and egg” argument — does biological aging promote atherosclerosis, or does atherosclerosis promote vessel aging and cellular senescence? We suggest that these scenarios are not mutually exclusive but both occur simultaneously. While both structural changes associated with aging and cellular senescence are associated with atherosclerosis, this review focuses predominately on the latter, particularly on VSMC senescence, although other cell types undergo the same processes after the same stimuli. We will outline evidence of cellular senescence, proposed mechanisms, as well as current therapeutic options targeting vascular aging or cellular senescence in atherosclerosis.

During fibrous plaque development, atheroma plaques undergo a transition from the fatty streak to intimal growing, a step characterized by the presence of a cell-free and lipid-rich area

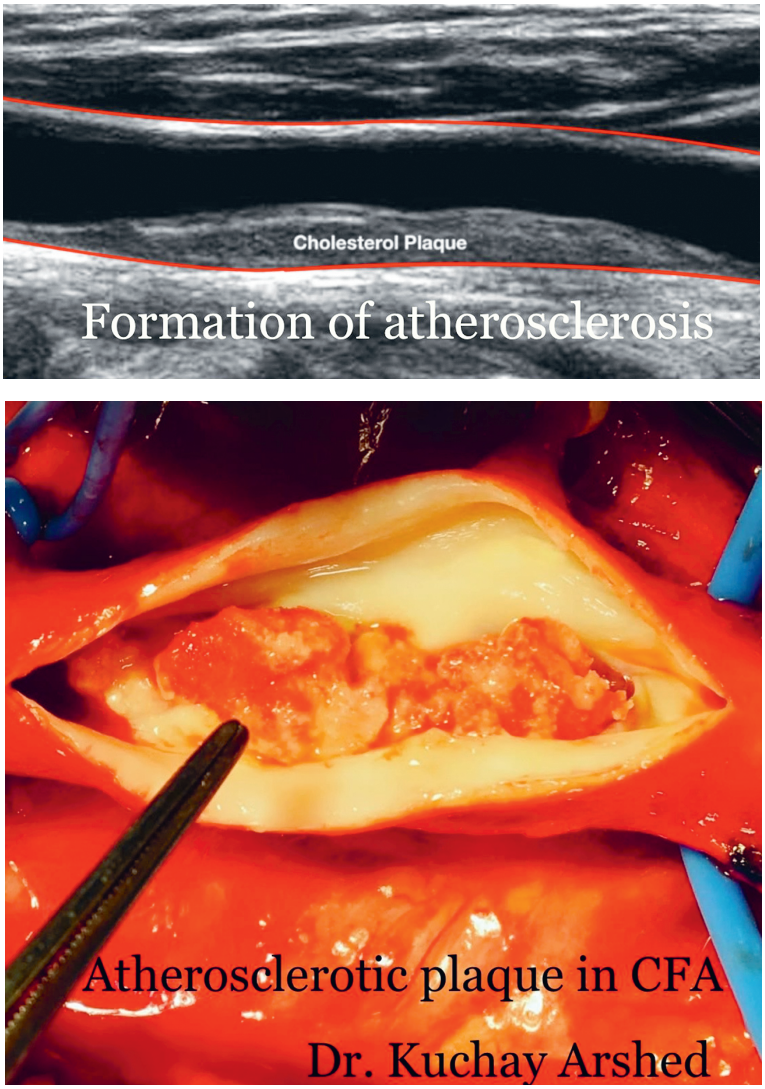


Рис. 4. Формирование бляшек в артерии

Fig. 4. Representation of plaque formation in artery

known as the necrotic core (Fig. 4). To stabilize the plaque, the necrotic core is covered by fibers, thus developing a fibrous cap. The necrotic core and the fibrous cap constitute the hallmark of advanced atherosclerosis [31], and atheroma plaque regression is unlikely to happen in this stage.

The fibrous cap is a subendothelial barrier between the lumen of the vessel and the atherosclerotic necrotic core consisting of VSMCs that have migrated to the luminal side of the artery and ECM derived from VSMCs. The role of the fibrous cap is to serve as a structural support to avoid the exposure of prothrombotic material of the core that otherwise would trigger thrombosis.

At the physiological situation, differentiated VSMCs of the tunica media show a contractile phenotype that regulates the blood vessel diameter and blood flow. However, in response to injury, VSMCs switch their phenotype to the synthetic one in which migratory and proliferation activities prevail [32]. For that purpose, neighboring cells activate the healing process by producing several growth factors, which include epidermal growth factor, fibroblast growth factor, insulin-like growth factor, platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), and vascular endothelial growth factor (VEGF) [32]. In atherosclerosis, in response to the growth factors produced by foam cells (VSMC- or macrophage-derived) or ECs of the intima, VSMCs from the tunica media migrate to the intima. Moreover, Interleukin-1 (IL-1) produced by macrophages enhances the endogenous production of PDGF by VSMC,

and, once in the intima, it autocrinically leads to their proliferation. In addition to migration and subsequent proliferation, VSMCs with a synthetic phenotype also increase the production of ECM components, such as interstitial collagen, elastin, and proteoglycans. These proliferating VSMCs, along with ECM production, generate a fibrous cap that will cover the developing atherosclerotic plaque, thus surrounding the lesion and preventing its rupture. Interestingly, if the mitogen production does not cease, VSMCs do not switch back to the contractile phenotype, which facilitates lesion development. Fibrous-cap features, such as thickness, cellularity, matrix composition, and collagen content, are important determinants of plaque stability.

There are marked changes in multiple cell types in older versus younger individuals, including VSMCs [49, 50], ECs [121], and inflammatory cells [122]. Prematurely and naturally aged cells share several characteristics, including changes in cell proliferative potential, markers of cell senescence, increased propensity to undergo cell death, elevated deoxyribonucleic acid (DNA) damage, and extensive telomere shortening and dysfunction. All of these features can be detected in cells from atherosclerotic plaques, which show additional features of cell senescence.

The necrotic core constitutes the nucleus of the atherosclerotic plaques. Covered by the fibrous cap, the necrotic core consists of a lipid-laden hipocellular region with reduced supporting collagen [33]. While atherosclerotic plaque develops, the necrotic core increases its size mainly as a consequence of two factors, macrophage death and impaired efferocytosis, a process that removes apoptotic cells. Both events contribute to an inflammatory microenvironment, oxidative stress, and thrombogenicity and promote the death of neighboring cells, such as VSMCs, increasing plaque vulnerability.

In the early stages of atherosclerosis, macrophage apoptosis is programmed through the coordinated caspase system, leading to cell death and efferocytosis. However, when atherosclerosis develops, apoptosis enhances in the macrophage and VSMCs due to increased oxidative stress, the activation of receptors involved in death signaling, the inhibition of survival pathways, and nutrient deprivation [33]. At this step, the phagocytic activity of the macrophages is not able to handle the accumulation of apoptotic cells, which undergo a secondary necrotic death with the concomitant release of intracellular oxidative and inflammatory components. This situation aggravates inflammation and enhances the death of neighboring cells. Efferocytosis also becomes defective in advanced atherosclerosis because the activity of several receptors that mediate the process, such as MerTK, LRP1, CD36, and SR-B1, is impaired. Moreover, the oxidized phospholipids and oxLDLs present in advanced atherosclerotic plaques inhibit the recognition of apoptotic cells by efferocytotic receptors. Efferocytosis impairment in advanced plaques also favors cholesterol crystal accumulation coming from apoptotic cells. At physiological conditions, small cholesterol crystals are rapidly sequestered from interstitial space by phagocytic cells; however, while the lesion advances, phagocytic cells are unable to remove the excess of crystals, which finally increase in size and remain in the subendothelial space. This process activates the complement and increases the proinflammatory state of the plaque. Moreover, as phagocytic cells are unable to internalize large cholesterol crystals by scavenger receptors, their lysosomal content is directly secreted to the interstitial space, promoting a more intense immune response. These events promote the death cell accumulation and necrotic core growth. Furthermore, the metalloproteinases released after cell death reduce the size of the fibrous cap and increase plaque vulnerability. Finally, apoptotic and necrotic cells liberate tissue factor (TF), which, along with oxidized lipids, increases the thrombogenicity of the necrotic core.

Atheroma plaque calcification is another hallmark of advanced atherosclerosis. It exists as a bone-like formation within the plaque and is initiated in inflammatory regions with a local decrease in collagen fibers. The release of matrix vesicles upon the macrophage and synthetic VSMC death initiates the calcification process of the plaque. The nucleation sites accumulate calcium orthophosphate, which is converted to amorphous calcium phosphate and finally to crystalline structures. In addition, other factors, including reduced levels of mineralization inhibitors or increased osteogenic trans differentiation, contribute to the calcification process. In particular, pericytes and VSMCs transdifferentiate into osteoblast-like phenotypes during atherosclerosis development, acquiring the capacity to generate a mineralized matrix and leading to calcium deposits, as it occurs in bone tissue.

Together, this contributes to microcalcifications, the early stage of the vascular calcification cascade in both intima and media. Microcalcifications then evolve into larger calcifications that extend from the bottom of the necrotic core to

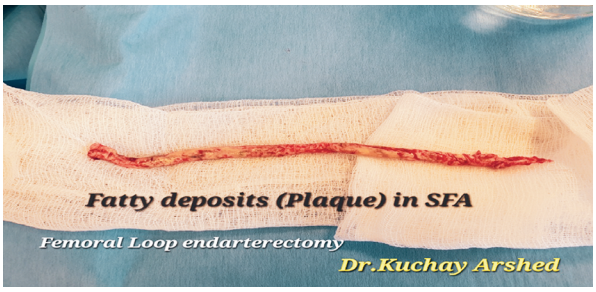


Рис. 5. Жировые отложения (бляшка) в поверхностной бедренной артерии (протяженная окклюзия)

Fig. 5. Fatty deposits (plaque) in superficial femoral artery (extended occlusion)

the surrounding matrix. Although calcification is a hallmark of advanced atherosclerosis (it correlates very well with plaque size), the amount and size of calcium deposits are not correlated with plaque vulnerability, which would rather be linked to other features, such as location, calcification type, or the surrounding environment (Fig. 5) [35].

CELL PROLIFERATION

VSMCs in the normal vessel wall have a very low turnover, with barely measurable proliferation indices. Increased cell proliferation is observed during early atherogenesis and on vascular injury [51] and aged VSMCs from rodents also show increased proliferation [49, 50, 52] compared with cells from younger animals. In contrast, human VSMCs derived from both aged vessels and advanced atherosclerotic plaques undergo reduced proliferation and prolonged population doubling times [53, 54]. This observation corresponds to *in vitro* findings in which plaque VSMCs in culture show decreased percentages in S-phase and increased percentages in G_1, consistent with a G_1 growth arrest [54]. While some of the arrest is associated with reduction in responses to mitogens, such as insulin-like growth factor 1 (IGF-1) [55], the arrest is mediated by major changes in the expression of various cell cycle regulators, especially those involved in G_1-S transition. Thus, increased expression of the cyclin-dependent kinase inhibitors $p16^{INK4a}$ and p21 [56], decreased cyclin D and cyclin E [57], and hypophosphorylation of the retinoblastoma protein (pRB) [57–58] are observed in both normal human VSMCs undergoing replicative senescence and human plaque VSMCs. Plaque VSMCs also show reduced expression of the transcription factors E2F1–3 and increased sequestration of E2F1 to pRB [56]. Importantly, these cell cycle regulators become potential markers of vascular cell senescence.

Although most of these studies have examined cells that are presumed to be derived from the vessel wall itself, a number of published works suggest that at least a proportion of cells expressing VSMC or EC markers may be derived from bone marrow–derived progenitor cells (BMCs) and endothelial progenitor cells (EPCs) that migrate and integrate into the vessel wall and may themselves be affected by aging [59, 60]. This is a very controversial area [61–63], but dysfunctional EPCs [64] and impaired BMC migration and adhesion [60, 64] are seen in both aged and atherosclerotic mouse models. These cells from aged animals are accompanied by reduced expression of cell surface markers and cytokines for chemotaxis, such as C-X-C chemokine receptor type 4 [60, 65], decreased hypoxia-inducible factor 1A [66, 67], as well as increased oxidative stress and inflammation [68, 69].

APOPTOSIS

Although cell death through multiple processes (necrosis, autophagy, apoptosis) occurs in atherosclerosis, the best described in association with senescence is apoptosis. Apoptosis, identified by morphological and molecular changes, is increased in aged vascular cells and is also increased in VSMCs and other cells in atherosclerotic plaques [51, 54]. While apoptosis occurs in ECs, VSMCs, and macrophages, most cell death in plaques is within the macrophage-rich necrotic core of the plaque. However, many apoptotic cells lose their lineage markers, which makes their precise identity difficult to be determined [51].

Aside from shortened telomeres, VSMCs, ECs, macrophages, and circulating cells from the elderly and patients with atherosclerosis contain increased DNA damage in both nuclei and mitochondria [70–73] compared with younger subjects and those without vascular disease. The accumulation of DNA damage in cells may reflect both ongoing damage-inducing stimuli and defects in the repair machinery. Similar to telomere shortening, DNA damage eventually leads to cell senescence and apoptosis, although minor damage is associated with transient growth arrest as repair is undertaken. DNA damage includes both single- and double-stranded breaks, deleted sections of DNA, nucleotide modifications, and extrusions of DNA from the nucleus (micronuclei), accompanied by altered expression of various damage response (DDR)

proteins [74]. In brief, the DDR is initiated by the identification of "faulty" DNA, either a mismatch base or single- or double-stranded breaks. Identification and signaling is achieved by recruiting various sensor proteins, including phosphorylated forms of ataxia telangiectasia mutated (P-ATM) and histone 2A protein X (γ-H2AX). Expression of both P-ATM and γ-H2AX increase with atherosclerotic plaque grade *in vivo*, and both markers are elevated in plaque VSMCs *in vitro* compared with cells from normal vessels [73]. P-ATM and γ-H2AX are therefore used as common markers to identify DNA damage and the DDR *in vivo*. Recruitment of these proteins triggers an intracellular kinase cascade leading to the activation of a range of effectors to participate in DNA repair, including p53 and Chk2. The normal response is transient cell cycle arrest and repair of the damaged DNA. However, when DNA damage is too extensive to be repaired or when the repairing cascades are impaired, cell senescence and apoptosis occur.

Oxidized LDL induces the endothelial cells to produce inflammatory mediators and to express adhesion molecules, this, in turn, will drive inflammatory process and immune cell infiltration at the lesion sites [22]. Oxidized LDL is implicated in the following pathogenic effects: upregulation of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), selectins and chemo attractants such as lipids, platelets-activating factor, and chemokines such as interleukin-8 and monocyte chemoattractant protein-1 (MCP-1) [29]. Circulating monocytes become adherent to the activated endothelial cells, this adhesion process will be followed by migration of monocytes by process of diapedesis through intercellular junctions to the subendothelial space [16]. Once monocytes reside in subendothelial space they acquire the macrophage characteristics under the effect of macrophage colony stimulating factor [23], and express dcavenger receptor (SR) [A, B1, CD36, CD68, lectin-like oxidized low density lipoprotein receptor-1 (LOX-1)] that will bind modified lipoproteins such as OxLDL, native lipoproteins and anionic phospholipids [21].

Once oxidation of LDL started, it amplifies itself and will be accompanied by monocyte recruitment and further macrophage production. One proposed mechanism for that is the binding of lipoproteins to proteoglycans which are secreted by macrophages this will further amplify and retain Ox-LDL, making lesion's inflammation as a continuous process [23]. Moreover, macrophages release inflammatory mediators, such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor α (TNFα) which are potent regulators of variety of cells involved in atherosclerotic plaque formation [32]. Interleukin-6 is a key cytokine in pathogenesis of atherosclerotic plaque development and progression [16, 17]. During the past three decades, extensive researches have been conducted in order to explore possible mechanisms by which Ox-LDL plays its role in fatty streak progression. One mechanism was probably through lysophosphatidylcholine a product of LDL oxidation, this product has been shown to be monocytes and T-lymphocytes chemoattractant factor and an inducer for endothelial cells to express the adhesion molecules VCAM-1 and ICAM-1 [35]. Lysophosphatidylcholine (LPC) found to be associated with increased levels of the platelets derived growth factor and heparin-binding epidermal growth factor in endothelial and SMCs, these two factors are potent attractant and inducers for SMCs proliferation this explains SMCs growth and migration, and platelets chemoattraction to the atherosclerotic plaque [18]. SMCs proliferation and migration from tunica media to tunica intima play an important role in growth and expansion of atherosclerotic plaque [14, 15]. Interestingly, Ox-LDL can induce metalloproteinase-1 (MMP-1) and metalloproteinase-9 (MMP-9) in the vascular wall matrix causing degradation of extracellular collagen leading to weak and unstable atherosclerotic plaque [19].

THE PATHOGENIC ROLE OF OXIDATIVE STRESS AND OXIDIZED-LOW DENSITY LIPOPROTEIN (OX-LDL) IN ATHEROSCLEROSIS

Oxidative stress is a biomedical term that was coined about three decades ago and further introduced into biomedical field and medicine since 1985. One of the best practical definitions of oxidative stress was given by Lushchak, who defined the oxidative stress as a situation of disturbed cellular metabolism or unregulated cellular function with damaged cellular constituents due to transient or chronic enhancement of reactive oxygen species (ROS) concentration [35]. ROS that play a key role in pathogenesis of atherosclerosis are superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), nitric oxide (NO) and peroxynitrite ($ONOO^-$) [19]. Recently, many articles and books have been published in concern with the topic oxidative

stress. The vast majority of those publications are in the field of medicine relating oxidative stress with the pathogenesis of some diseases such as atherosclerosis, cancer and neurodegenerative disorders. The deleterious effect of ROS on blood vessels and on biological system depends on their amount and on efficacy of antioxidant system. If ROS formed intracellularly in low amount they function as the second messenger for fibroblasts and SMCs growth, in addition to their role in the immune system, but if they are produced in large amount they can cause cytotoxicity, DNA damage and apoptosis [19]. The antioxidant system includes antioxidant enzymes (e.g. superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and reductase, etc.), nutrient derived antioxidants (e.g. ascorbic acid, tocopherols and tocotrienols, carotenoids, glutathione and lipoic acid), metal-binding proteins (e.g. ferritin, lactoferrin, albumin and ceruloplasmin) and numerous other antioxidant phytonutrients present in a wide variety of plant foods [23]. Whenever the balance between ROS production and antioxidant defense is lost, "oxidative stress" results leading to various pathological conditions. Local arterial wall or systemic oxidative stress is one of the proposed mechanisms for development and progression of atherosclerosis [18].

There is evidence by *in vitro* and by experimental as well as by human studies that oxidized lipids derived from LDL contribute to multiple stages of atherosclerotic plaque development and progression through production of inflammatory cytokines (e.g. TNFα), upregulation of (NADPH) oxidases and increase in renin-angiotensin system. Animal models fed high cholesterol diet have provided the first experimental evidence for the role of LDL in oxidative stress [16, 17]. Before that, there was evidence for the role of ROS O_2^- in lipid peroxidation, as demonstrated by invitro study showed O_2^- generated by vascular SMCs was responsible for oxidative modification of LDL. Due to accumulative formation of Ox-LDL in the atherosclerotic lesions, oxidative stress through Ox-LDL plays its pathological role by acting as potent stimulus for ROS production within the vascular wall. Once Ox-LDL engulfed by macrophages via SR it will contribute to formation of foam cells in vascular intima and propagates further oxidative stress [21]. Oxidized LDL induces ROS production through which it contributes to further foam cells formation at atherosclerotic plaques. Most of ROS produced at vascular endothelium are due to activation of NADPH oxidase following binding of Ox-LDL to macrophage LOX-1 receptors. The NADPH-oxidase mechanism has been considered as the abundant operating oxidative stress mechanism in atherosclerosis. Furthermore, Ox-LDL will activate macrophage signaling pathway through CD36 SR, leading to more ROS production which mediates secretion and maturation of IL-1β, subsequently IL-1β will promote macrophage cell formation by autocrine function [15].

Diet and dietary habits are major driving forces for development and modification of atherosclerotic diseases. Diet and intestinal bacteria (microbiome) are implicated in the pathogenesis of atherosclerosis. This approach was introduced after discovery of the role of metabolic factors other than dietary cholesterol [30]. Phosphatidylcholine found in egg yolk and in red meat carnitine is processed by intestinal bacteria to trimethylamine (TMA) this in turn oxidized in the liver to trimethylamine N-oxide (TMAO) which has been described in animal models and clinical trials as metabolite causing atherosclerosis and elevating risk of CHD [22]. Trimethylamine N-oxide found to promote formation of foamy macrophages (hall mark of fatty streak) [16]. Dietary choline or TMAO supplementation increases the expression of the SR on macrophages and reduces reverse cholesterol transport, promoting foam cell formation. Precursors of TMAO, choline or L-carnitine elicit alterations in cholesterol and sterol metabolic pathways in the vascular wall [23]. People such as vegans lacking intestinal bacteria that can produce trimethylamine; hence, they may be protected against atherosclerosis. Level of TMAO can be influenced by the type of diet, variations in expression of flavin monooxygenases (enzymes that convert TMA to TMAO) and perhaps the composition of gut flora [16]. This principle has raised a new approach for treatment of atherosclerosis through eradication of pathogenic bacteria with antibiotics and recolonization via stool transplantation with beneficial bacteria, but this novel work still under investigation.

Atherosclerosis is a multifactorial arterial disease involving interactions of multiple genetic and environmental factors. Majority of our understanding of the factors contributing to atherosclerosis has come from epidemiologic studies and from studies of Mendelian forms of the disease, such as familial hypercholesterolemia. These have revealed important roles for plasma lipoprotein, blood pressure, diabetes and other risk factors [16]. Genetics have a significant influence on susceptibility to atherosclerotic vascular

diseases. This fact proven through the continuous advances in molecular genetic techniques. The first evidence about role of genes contributing to atherosclerosis was provided by genome wide association studies (GWAS) [16]. Interestingly, unbiased GWAS have identified a number of novel loci robustly associated with atherosclerotic CHD. Recently, an abundance of candidate genes, genetic polymorphisms and susceptibility loci associated with atherosclerotic diseases has been identified. A recent ongoing effort using bioinformatics-based approaches resulted in 98 new candidate genes for CHD. One example of such approach was done by 67, who generated 63 chromatin interaction data sets for 37 active DNA regulatory elements that colocalize with known susceptibility loci for coronary artery disease (CARDIoGRAMplusC4D) and large artery stroke (METASTROKE). On the other hand, epigenome-wide association studies (EWAS) identified a set of 84 genes highlighting the relevance of obesity, inflammation, lipid and carbohydrate metabolism in atherosclerotic CHD. Epigenetic means chemical tags that modify DNA expression independent of the DNA sequence. In other words, epigenetic modifications can alter the expression of genes without changing their sequences [22]. Common types of epigenetic modification include DNA methylation, histone modification and ribonucleic acid (RNA)-associated silencing. Methylation of DNA in the genome was found to play a major role in the development and progression of atherosclerosis. Enzymes which influence DNA methylation such as DNA methyltransferases (DNMTs) are crucial in maintaining endothelial cell integrity, promoting SMC proliferation which are implicated in pathogenesis of atherosclerosis [22]. This approach may be utilized to develop new diagnoses and treatments for atherosclerosis-related diseases. DNA methylation has been linked to oxidative stress and inflammatory processes underlying pathogenesis of atherosclerosis. Diversity of studies have shown that oxidative stress affects DNA methylation and hence ROS influence pathogenesis of atherosclerosis [22–24]. DNA methylation is an important mechanism that regulates gene expression associated with inflammation and atherosclerosis. Alterations in DNA methylation have also been implicated in diseases related to chronic inflammation. Recently, DNA hypermethylation has been linked to inflammation and found to cause increased mortality in atherosclerosis-related diseases [22]. Histone acetylation and methylation have been reported to play a decisive role in atherosclerosis [26]. Despite evidence-based role of genetics and epigenetics in the pathogenesis of atherosclerosis, future molecular studies in these fields are recommended to interrelate candidate genes, genetic polymorphisms and susceptibility loci for better mechanistic understanding of atherosclerosis pathogenesis. Moreover, functional assays are required to understand the molecular mechanisms that relate DNA methylation to atherosclerosis and to determine its causal or reversal of causality role aiming for future therapeutic options.

FUNCTIONAL CONSEQUENCES OF CELL AGING

Aging-associated changes are observed in multiple cell types and are conserved across species, from rodents to primates. As well as loss of normal cellular function, these changes result in specific consequences in each cell type, which may be directly proatherogenic [113].

Aged ECs become flatter, enlarged, and have an increasingly polypoid nucleus, all features associated with cellular senescence [75–78]. These changes are accompanied by modulation in cytoskeleton integrity, proliferation, angiogenesis, and cell migration. Senescent ECs show attenuated endothelial NO production [79] and increased endothelin-1 release [80]. Late-passage ECs also show reduced expression of the adhesion molecules VCAM-1 and intracellular adhesion molecule-1 (ICAM-1), increased activation of nuclear factor (NF)-κB, and increased susceptibility to apoptosis [78]. Moreover, there are marked age-associated changes in ICAM-1 function and activity [81]. Thus, EC senescence is associated with loss of EC function and a shift toward a proinflammatory and proapoptotic state, predicted to enhance monocyte migration into the vessel wall.

VSMCs in human plaques or derived from plaques show reduced proliferation, early senescence, and increased susceptibility to apoptosis. These properties would reduce the ability to repair plaques that undergo rupture. Aged rodent aortas also show increased levels of IL-6 and aged aortic VSMCs have a higher basal secretion of IL-6 than young VSMCs. Indeed, secretion of a common set of secreted proteins as cells age is a widespread phenomenon known as the “senescence-associated secretory phenotype”, or SASP. Moreover, aged VSMCs exhibit upregulation of chemokines (chemokine CC-motif ligand 2), adhesion molecules (e.g., ICAM-1), and innate immune receptors (e.g., Toll-like receptor 4 [82]. These properties generate a proinflammatory

environment, further promoting migration of inflammatory cells. VSMC senescence thus promotes atherosclerosis progression and inhibits plaque repair.

Monocytes from patients with atherosclerosis demonstrate an increased burst of free radicals on activation and increased secretion of a number of cytokines, including monocyte chemotactic protein (MCP) -1, IL-6, IL-1β, and tumor necrosis factor-α [83]. Importantly, many of these differences are also observed in aged versus young monocytes and can be recapitulated by agents that disrupt telomeres [83]. Thus, there is direct evidence that aging promotes proinflammatory changes in monocyte/macrophages that are relevant to atherosclerosis [83]. Coupled with altered adhesion molecules on aged ECs, aging would be predicted to promote both migration and activation of macrophages within plaques.

Cell senescence is defined as the irreversible loss of the ability of cells to divide. There are 2 general types of cell senescence, replicative senescence and stress-induced premature senescence (SIPS). Replicative senescence occurs with exhaustion of proliferative lifespan over time, a characteristic of aging, and is associated with critically shortened telomeres at chromosomal ends, which then induce a DNA damage response (DDR) (see below). In contrast, SIPS is triggered by external stimuli, including oxidizing agents and radiation, which activate the intracellular senescence cascade prematurely. While SIPS shares many morphological and molecular characteristics to replicative senescence, SIPS is not usually characterized by telomere shortening.

As well as altered expression of cell cycle regulators, senescent cells are characterized by "specific" markers, including senescence-associated β galactosidase (SAβG), a lysosomal enzyme seen in senescence of multiple human cell types [123]. Increased numbers of SAβG-positive VSMCs, ECs, and monocyte/macrophages are observed in aged vessels and atherosclerotic lesions when compared with their respective young and normal counterparts [56, 124], reinforcing the idea that atherosclerosis is associated with premature cellular senescence. However, a word of caution is required when interpreting SAβG staining. In particular, cells with a high lysosomal content, such as macrophage foam cells, show SAβG reactivity that may not reflect senescence.

TARGETING AGING IN ATHEROSCLEROSIS

The association between aging and atherosclerosis and the increasing evidence demonstrating that accelerated cellular senescence occurs in vascular disease have resulted in the search for treatments that can promote longevity and delay senescence. Indeed, pharmaceutical companies are now developing drugs that target specific mechanisms of premature aging, including telomerase activity, DNA methylation, DNA damage repair, and micro ribonucleic acid (miRNA). Specific agents may also target the DNA damage machinery and DDR directly. For example, chloroquine has been demonstrated in animal models to reduce atherosclerosis through a p53-dependent ataxia-telangiectasia mutated (ATM) signaling pathway [84]. Similarly, pioglitazone, a peroxisome proliferator-activated receptor agonist, can increase telomerase activity, TRF-2 expression, and phosphorylation of Akt and reduce the expression of the senescence markers p16, cell-cycle checkpoint kinase 2, and p53 [85].

Currently, it is debatable whether telomere length and telomerase are targets in atherosclerosis. As described above, although atherosclerosis is associated with telomere shortening in multiple cell types, apart from VSMCs [56] there is a paucity of evidence to demonstrate that shortening occurs to critical lengths that impair function. There is also only a little evidence [83] to show that the telomere shortening *in vivo* makes leukocytes proatherosclerotic. Furthermore, there is minimal evidence to suggest that telomere shortening initiates atherosclerosis rather than being a feature of advanced plaques. Although the lifespan of VSMCs and ECs can be extended by ectopic expression of telomerase, it is not clear if this is due to effects only on telomere length, and the fact that plaque VSMCs cannot be extended indefinitely indicates that plaque VSMCs have other causes for their senescence [56]. In addition, as described above, the mouse studies of telomerase manipulation in atherosclerosis are contradictory. Finally, and most importantly, increasing telomerase expression systemically has considerable risks. Agents that inhibit telomerase are being developed for cancer, [86] indicating that augmenting telomerase has the potential to be carcinogenic and can add the undesirability of uncontrolled proliferation of VSMCs. Therefore, much more research must be undertaken in this area before manipulation of telomere length or telomerase activity as a primary mode of action could be considered for therapeutics in atherosclerosis, although agents that indirectly promote telomere stability in cells on the vessel wall may still be beneficial.

In addition to more specific mechanisms, a number of currently available drugs and compounds are likely to delay premature aging as part of their mechanisms of action through changes in ROS and oxidative DNA damage. Examples of these include antioxidants, statins, and angiotensin-converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARBs). Dietary manipulation and augmentation of sirtuin activity also have potential to reduce vascular aging, cellular senescence, and atherosclerosis.

Expression of naturally occurring antioxidants including ferritin and glutathione are reduced with age and in atherosclerosis [87, 88]. Coupled with evidence of increased ROS in atherosclerosis and the demonstration that ROS promote premature aging, this has resulted in intensive study of the use of antioxidants to prevent cardiovascular diseases and aging. However, although antioxidants have been demonstrated to have significant antiatherosclerosis effects *in vitro* and in animal models, their efficacy in humans is questionable. For example, Vitamin E can reduce the uptake of oxLDL by macrophages and inhibit the subsequent inflammatory responses that stimulate atherosclerosis development [89, 90]. Also, antioxidants can modulate epigenetic patterns through regulating levels of methyl donors and methyltransferase inhibitors [91]. However, there is now extensive evidence indicating that supplementing dietary antioxidants has no significant effect on reducing cardiovascular risk [92, 93].

Although there may be many reasons why antioxidants might not reduce atherosclerosis, the therapeutic failure of dietary supplementation has simulated interest in enhancing natural antioxidant pathways. For example, overexpression of nuclear factor E2 — related factor-2 (Nrf2), a key transcription factor orchestrating antioxidant and cytoprotective responses, in VSMCs and ECs reduces oxidative stress and attenuates inflammatory responses [94, 95]. Nrf2 activators such as Protandim can increase the production of multiple antioxidant enzymes [96]. In contrast, Nrf2 knockout mice develop smaller atherosclerotic plaques with improved arterial stiffness, regardless of increased serum cholesterol and lipid oxidation [97]. This phenotype is achieved, at least in part, through downregulation of the CD36 receptor required for oxLDL uptake by macrophages, which reduces macrophage apoptosis and the subsequent inflammatory responses. These findings demonstrate that Nrf2 mediates multiple pathways independent of its antioxidant effects, and more studies are required before using Nrf2 as a therapeutic option for atherosclerosis [125–135].

The hydroxy-methylglutaryl-coenzyme A reductase inhibitors (statins) reduce cholesterol synthesis and hence reduce atherogenesis, the progression of established plaques, and cardiovascular risk. Although their major effect may be via cholesterol reduction, statins have multiple effects that are not necessarily associated with their primary mechanism, including improved endothelial function and reduced inflammation responses. More specifically, statins target mechanisms inducing premature aging, leading to enhanced telomere protection through upregulating TRF2 [100], decreased DNA damage by accelerating DNA damage repair [73, 101], and suppressing oxidative stress in part by increasing antioxidant defenses [102]. Statins can also delay VSMC replicative senescence and reduce markers of DNA damage *in vivo* in atherosclerosis [73].

Angiotensin II increases ROS and promotes oxidative DNA damage, promoting senescence through telomeric and nontelomeric DNA damage [103]. Whereas ACE inhibitors/ARBs have multiple cardiovascular effects, they can also reduce oxidative stress and subsequent DNA damage. At present, it is not known whether these drugs reduce premature aging *in vivo* and whether this is an important mode of their action. However, bradykinin, a hormone that mediates some of the vasoprotective effects of ACE inhibitors, protects ECs from superoxide-induced senescence through bradykinin B2 receptor-mediated and NO-mediated inhibition of DNA damage [104].

Nutritional status and diet are associated with age-related vascular changes and with atherosclerosis [105]. In particular, caloric restriction (CR) is a common method to manipulate diet and the beneficial effects of CR on vessel aging have been proven in animal models [106, 116, 107], including preservation of matrix components within the vessel wall [108], improving EC function through augmenting NO generation [109], reducing sensitivity to oxLDL [110], reducing oxidative stress by upregulating antioxidants and protecting mitochondria function [109, 111], and inhibiting inflammation [112]. Although CR may be inappropriate in many patients, drugs and dietary supplements that mimic CR effects without affecting nutritional balance may offer a wider therapeutic option.

Sirtuins (SIRT1–7) are a family of nicotinamide adenine dinucleotide $(NAD)^{+}$-dependent deacetylases and adenosime diphosphate–ribosyltransferases that may be partially responsible

for the age-delaying effects of CR. Sirtuins have been reviewed as part of this series [113]; therefore only limited discussion is provided here, focusing on SIRT1, the most well-studied member. CR increases SIRT1 in some experimental models, leading to improved endothelial function [107, 116] while knocking down SIRT1 interferes with the CR-mediated antioxidant and anti-inflammatory vascular effects [112]. Similar to CR, overexpression of SIRT1 in the endothelium can improve vascular stiffness and attenuate the development of atherosclerosis [114], probably by activating endothelial nitric oxide synthase (eNOS) and promoting NO production [114, 115] and preventing EC senescence [117]. Indeed, SIRT deacetylation of eNOS may contribute to the atheroprotective effects of laminar stress [118]. SIRT1 in hematopoietic cells also prevents foam cell formation and reduces atherosclerosis [119]. The agent Resveratrol, which can increase SIRT1 expression, reduces EC apoptosis and increases aortic elasticity in aged rodents [120], although how much of this effect is due to SIRT1 is unclear. Moreover, other sirtuin members, such as SIRT3–5, as sensors of nutritional status may be protective in vascular system, regulating the cellular response to stress, energy production, apoptosis, and ROS production [98, 99].

CONCLUSIONS

Atherosclerosis is a chronic inflammatory disease of the inner wall of large- and medium-sized arteries. Its basic pathogenic mechanisms are inflammation and oxidative stress involving interactions with multiple genetic, epigenetic and environmental factors. The LDL-c and its oxidative modified forms contribute to multiple stages of atherosclerotic plaque development through production of inflammatory cytokines. Understanding of pathogenic role of genetics, epigenetics, inflammatory and immune mechanisms in atherosclerosis at molecular level may be attractive targets for disease prevention and/or treatment.

The normal vascular aging and atherosclerosis are associated with cellular senescence. Cellular senescence impairs cell proliferation resulting in irreversible growth arrest and impairs survival, due to an accumulation of nuclear and mitochondrial DNA damage, increased prionflammatory state and ROS. Both cellular senescence and vascular aging are associated with increased expression of prionflammatory cytokines and adhesion molecules further promoting inflammation and also affect the synthesis and maintenance of extracellular matrix proteins. Aging can be identified by both structural changes and by a number of senescence-associated biomarkers. Advanced atherosclerosis is likely to manifest irreversible changes, prevention of accelerated cell aging becomes a major therapeutic opportunity. Understanding the mechanisms contributing to such changes is therefore crucial for both the prevention and the development of treatment for atherosclerosis and other age-related diseases.

During the last few years, we are witnessing an increased burden of atherosclerotic disease that contributes to CVD risk, which is becoming a global epidemic. The study of cellular and molecular biology mechanisms of atherosclerosis has provided remarkable insights into the processes that lead to atheroma development and the clinical manifestations of this disease. Knowledge and continued research about the functions of non-coding RNAs in plaque development have improved, showing that miRNAs and lncRNAs alter the transcription of genes implicated in atherosclerosis. In addition, microbiota have been linked to the development of atherosclerosis by identifying microbial ecosystems residing in different habitats of the human body that contribute to metabolic and cardiovascular disorders. The role of microbiota in atherosclerosis development is supported by increasing mechanistic evidence; however, further studies are needed to understand the contribution of microbiota to atherosclerosis. Finally, the importance of analyzing sex-specific differences as risk factors associated with atherosclerosis is important for individualized risk-management strategies to prevent the development and progression of atherosclerosis. The progress in understanding the mechanisms that lead to atherosclerosis development will surely provide therapies to address the unacceptable burden of persistent risk and will ultimately improve the diagnosis and prognosis of the disease.

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ADDITIONAL INFORMATION

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