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Review

The carotid plaque as paradigmatic case of site-specific acceleration of aging process: The microRNAs and the inflammaging contribution

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ABSTRACT

Atherosclerosis is considered a chronic inflammatory disease of arteries associated with the aging process. Many risk factors have been identified and they are mainly related to life-styles, gene-environment interactions and socioeconomic status. Carotid and coronary artery diseases are the two major atherosclerotic conditions, being the primary cause of stroke and heart attack, respectively. Nevertheless, carotid plaque assumes particular aspects not only for the specific molecular mechanisms, but also for the types of atheroma which may be associated with a better or a worst prognosis. The identification of circulating blood biomarkers able to distinguish carotid plaque types (stable or vulnerable) is a crucial step for the improvement of adequate therapeutic approaches avoiding or delaying endarterectomy in the oldest old individuals (> 80 years), a population predicted to growth in the next years. The review highlights the most recent knowledge on carotid plaque molecular mechanisms, focusing on microRNAs (miRs), as a site-specific accelerated aging within the conceptual framework of Geroscience for new affordable therapies.

1. Introduction

Human aging is a dynamic lifelong process which is sustained by a chronic low grade pro-inflammatory tone we dubbed inflammaging (Franceschi et al., 2000). Stimuli able to fuel inflammaging include a variety of molecular garbage ("garbaging") that may accumulate and propagate, as we recently proposed (Capri et al., 2020; Franceschi et al., 2017). In fact, many inflammatory misplaced/unfolded molecules - as result of cellular engulfment or cell debris- may propagate to proximal or distal cells, tissues, and organs, thus causing new foci of inflammation and becoming also traceable at systemic level.

In principle, each cell/organ/tissue and system of the body is differently affected by the aging process, resulting in a kind of age-related body mosaicism (Cevenini et al., 2008) due to different molecular mechanisms including the accumulation of genetic somatic mutations in mitochondrial DNA (mtDNA) (Rose et al., 2010), as well as mutations at genome level (Yizhak et al., 2019). The complexity of this mosaic is influenced by the interaction of several factors, such as life styles/environments, genetic-epigenetic relationship, which differ in the various cells of every organ/tissue.

The physiological process of aging and the molecular mechanisms causing age-related diseases and geriatric syndromes (ARD-GSs), such as type 2 diabetes (T2D), neurodegeneration (Alzheimer and Parkinson's Diseases, dementia), osteoarthritis, sarcopenia and frailty among others, have recently been proposed to be largely the same, highly interconnected molecular pillars/pathways (Kennedy et al., 2014). We highlighted that a continuum exists between aging and ARDs and the above-mentioned pillars converge on inflammation/inflammaging (Franceschi et al., 2000, 2007) contributing to accelerate the aging process itself (Franceschi et al., 2018a).

According to the World Health Organization (WHO) an estimated 17.9 million people died from cardiovascular diseases (CVDs) in 2016,

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representing 31 % of all global deaths. Of these deaths, 85 % are due to heart attack and stroke and over three quarters of CVD deaths take place in low- and middle-income countries (World Health Organization, 2017).

Similarly, in 2017, the leading world causes of death were ischemic heart disease and stroke at first and third position, respectively (Roth et al., 2018). In this scenario, atherosclerosis, a chronic inflammatory disease of arteries (Ross, 1999), plays a prominent role both locally and systemically. Atherosclerosis has a long, slow asymptomatic phase, starting early in life, several years before the onset of clinical signs (Libby et al., 2019; Rea et al., 2018) and in many patients becoming manifest at a relatively advanced age. Thus, aging is the most important atherosclerosis risk factor (North and Sinclair, 2012) even if smoking (de Weerd et al., 2014), high blood pressure (Bots et al., 1992; Mathiesen et al., 2001; O'Leary et al., 1992), dyslipidemia (Bots et al., 1992; Joosten et al., 2012; Mathiesen et al., 2001; O'Leary et al., 1992; Slovut and Olin, 2004), obesity (Kotsis et al., 2006) and T2D (de Weerd et al., 2014) also play a role.

Understanding the mechanisms involved in the age-related impairment of the vascular system is essential for reducing cardiovascular morbidity/mortality. A major source of preventable cerebral infarction is linked to large vessel atherosclerotic disease and specifically to internal carotid artery stenosis. In fact, the advanced knowledge of carotid plaque development, the current monitoring of patients and the accessibility to surgical intervention, *i.e.* endarterectomy, are crucial preventative opportunities. An estimation of 41,000 strokes annually may be attributed to extracranial internal carotid artery stenosis in the United States, (Flaherty et al., 2013) and a similarly, a huge number of strokes are also estimated in Europe (Naylor and Ricco, 2018). This scenario suggests that it is urgent to address the unmet needs of this topic by updating and improving both the preventative and the intervention guidelines on such vascular pathologies.

Accordingly, the present review will focus carotid atherosclerosis, a topic whose importance is relevant owing to the increasing number of old and very old patients for whom the decision to perform or not endarterectomy is the most difficult/critical. Thus, the identification of specific circulating blood biomarkers associated with the type of plaque (stable *vs* vulnerable) in old patients would be very useful to avoid surgery-related side effects. Furthermore, the most recent knowledge in terms of age-related epigenetic and molecular changes will be discussed focusing on microRNAs (miRs) since they are not only modulators of gene expression, but also biomarkers for new affordable therapies.

In particular, the conceptual framework of the Geroscience (Kennedy et al., 2014) including inflammaging will be exploited for a new perspective of the disease. The seven molecular pillars of Geroscience, indicating the shared molecular pathways among aging and aging-related diseases, will be analyzed in the context of carotid disease development.

2. Atherosclerosis and the case of carotid artery

Structural and functional changes occur with age both in central and peripheral arteries, as suggested by clinical and preclinical data (Rubio-Ruiz et al., 2014). Arterial stiffness increases (Donato et al., 2018; Scuteri et al., 2008) due to: i) augmented conducted wave velocity or changes in the passive mechanical properties of the artery that reduces its compliance; ii) molecular and cellular alterations of artery wall, leading to impaired endothelial function, chronic vascular inflammation, and calcification (Libby et al., 2019; Vasuri et al., 2014). All these modifications result in a complex dysregulation including microvascular perfusion and alterations of the extracellular matrix along with the effects of inflammatory and atherogenic responses. On the whole, an artery-specific accelerated aging process is in place. Risk factors such as life-style, gene-environment interaction (accompanied by epigenetic changes) and socioeconomic status are reported to contribute to arterial stiffness and atherosclerosis. In particular, the last seems to have a towering role being related to life-style and education, as recently shown (Gebreab et al., 2012; Nash et al., 2011; Thurston Rebecca et al., 2014).

Arteries differ regarding their propensity to develop atherosclerotic plaques, hereinafter plaque, owing to different/specific anatomical, histological, hemodynamic and metabolic features involved in artery wall damage (Tomas et al., 2018; Uslu et al., 2016). In particular, carotid and coronary artery diseases are the two major atherosclerotic conditions, being the primary cause of stroke and heart attack, respectively. These two arteries share similar mechanisms regarding the plaque formation but differences exist regarding risk factors, plaque biology, metabolic features (Tzoulaki et al., 2019) and progression, as recently and extensively reviewed (Sigala et al., 2018).

The anatomical details of the common carotid artery and its branches have attracted clinicians and researchers for their key role in the development of plaque (Uslu et al., 2016) and the stroke associated risk. The early lesions of atherosclerosis consist of subendothelial accumulation of cholesterol-engorged macrophages, *i.e.* the well-known foam cells, which contributes to the inflammatory process assumed as the basic pathogenic component in the development and progress of atherosclerotic disease (Bentzon et al., 2014; Donato et al., 2018; Libby et al., 2019; Lusis, 2000). The possibility to monitor the development and the stability of carotid plaque is clinically relevant especially for asymptomatic and elderly patients. On the other side, symptomatic patients are those subjects who have manifested signs of prior cerebrovascular events, such as transient ischemic attack, ischemic stroke and transient monocular vision loss, and urgently need of a surgical intervention at carotid artery. In this respect, the analysis of blood inflammatory cells (see below) and molecules, such as high-sensitivity C-reactive protein and serum amyloid A (Schillinger et al., 2005) may lead to a better evaluation of the patients' status, and particularly the identification of those subjects at higher risk of stroke recurrence (Marnane et al., 2014). Indeed, cytokines are crucial players being involved in all stages of the atherosclerosis pathogenesis (Ammirati et al., 2015; Hermus et al., 2010), but the scenario is highly complex as some cytokines, such as TGF-beta, IL-10, IL-13 and IL-33, may have anti-atherogenic effects (Moss and Ramji, 2016; Ramji and Davies, 2015). Cytokines also modulate endothelial cell permeability and recruit monocytes and T-lymphocytes in response to the increase of low-density lipoprotein (LDL), hypertension, and related shear stress (Ait-Oufella et al., 2011; McLaren et al., 2011).

A burning question is if the presence of comorbidities, predicted to increase in the next decades owing to the increasing number of old people, may accelerate the development and the progression of plaque (Ferrucci and Fabbri, 2018).

It is interesting to note that inflammaging may be accelerated by age-related comorbidities (Franceschi et al., 2018a), favoring atherosclerosis onset and plaque progression acceleration. In fact, recent data suggest that chronic kidney disease (Valdivielso et al., 2019) and T2D increase the prevalence of subclinical/asymptomatic atherosclerosis with a higher plaque burden (Palanca et al., 2018). The association of T2D with carotid plaque is particularly multifaceted especially in the framework of T2D complications, as patients frequently have comorbidities involving micro-macro-circulation. Calcification is also a well-recognized complication of atherosclerotic lesions in diabetic patients, which correlates with increased plaque burden, as recently reviewed (Yahagi et al., 2017).

At variance, some T2D studies suggest that carotid plaque morphology does not undergo burden progression (Scholtes et al., 2014) in diabetic patients, and diabetes does not represent *per se* a predictor for intima-media thickness progression (Herder et al., 2012). Such apparently conflicting results deserve further evaluation considering all the involved variables, including different therapies, presence/absence of complications or chronic diseases and comorbidities, all conditions that can contribute to systemic inflammation and modulate the central role of pro-inflammatory cytokines (Stankovic et al., 2019).

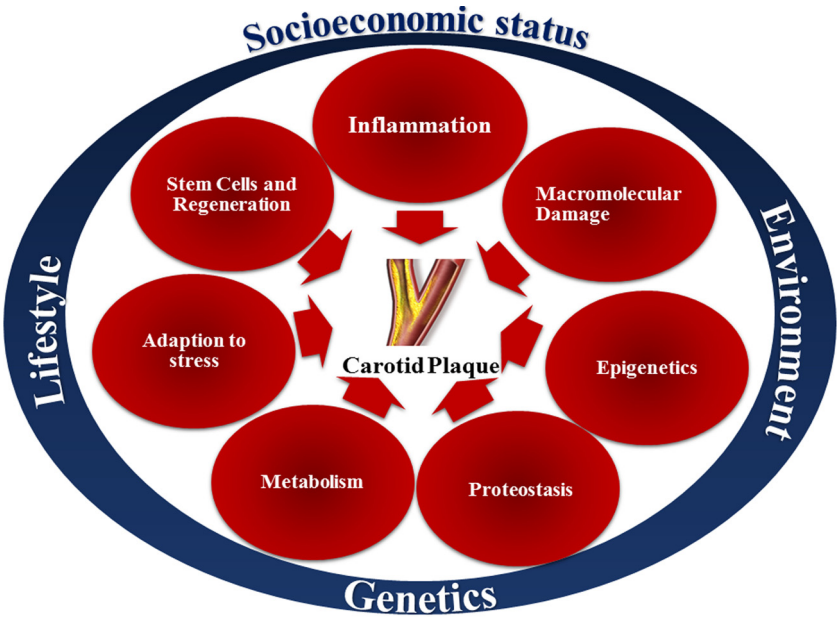

Fig. 1. The seven pillars of aging process within human carotid plaque. The main references related both to the seven pillars and the mechanisms of plaque development are listed in Table 1.

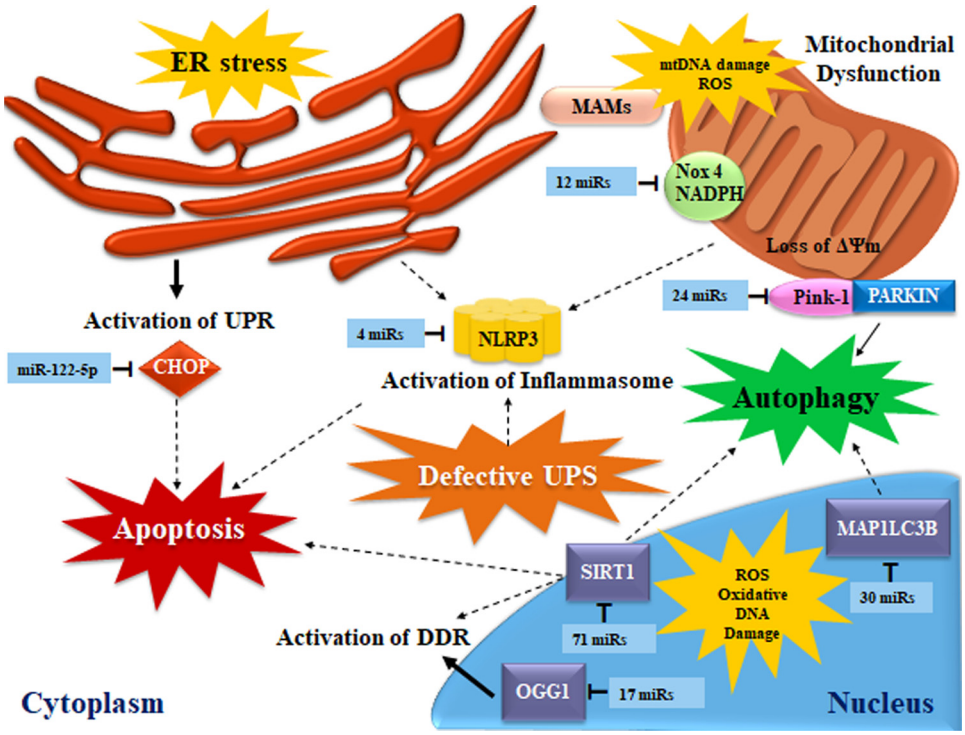

Fig. 2. The scheme of the molecular mechanisms involved in carotid plaque development. Oxidative stress, reactive oxygen species (ROS), mitochondrial dysfunction, defective disposal of misfolded and/or oxidized proteins by the ubiquitin/proteasome system (UPS), autophagy and DNA damage response (DDR), and endoplasmic reticulum (ER) stress, leading to the activation of the unfolded protein response (UPR) are shown. The miRs cited in the picture are listed in Table 1S.
Dashed arrows suggest the involvement of many steps. Abbreviations: CHOP: C/EBP homologous protein, ER: endoplasmic reticulum, DDR: DNA damage response MAMs: Mitochondria-associated ER membranes, MAP1LC3B: Microtubule-associated proteins 1A/1B light chain 3B, NLRP3: NLR family pyrin domain containing 3, OGG1: 8-Oxoguanine glycosylase, ROS: reactive oxygen species, SIRT1: Sirtuin 1

3. Carotid plaque: a site-specific acceleration of aging process

The basic molecular mechanisms of carotid plaque are well characterized and may be summarized in the seven pillars of Geroscience (Fig. 1), even if a different contribution for each pathway can be predicted.

The early damage of carotid involves various cells of artery wall transforming the site of injury in a peculiar microenvironment or niche (Tabas and Lichtman, 2017). In particular, vascular smooth muscle cells (VSMCs) show an increase of NOX4 NADPH Oxidase-Dependent mitochondrial oxidative stress correlated both with age (Donato et al., 2018) and the level of lesion (Vendrov et al., 2015) in human carotid. An increase of nuclear DNA (Martinet et al., 2002; Matturri et al., 2001) and mitochondrial DNA damage, together with a reduced DNA repair capacity (Shah et al., 2018) and telomere impairment (Matthews et al., 2006), have also been found in the same cells. Moreover, a subset of VSMCs within the plaque fibrous cap undergoes a mitochondrial dysfunction due to a decreased oxidative phosphorylation and the expression of Pink1 kinase which affect the site-specific metabolism increasing the glycolytic activity (Docherty et al., 2018). Thus, VSMCs may favor the set up of a pro-inflammatory niche.

In turn, the mitochondrial stress/damage within the lesion area induces a cascade of effects, including endoplasmic reticulum (ER) stress (Garbin et al., 2014; Tabas, 2010), ubiquitin-proteasome system

deregulation (Herrmann et al., 2008; Marfella et al., 2006; Versari et al., 2006) and defective autophagy (Alloza et al., 2016; Liu et al., 2015; Swaminathan et al., 2014).

These mechanisms may converge on NLP3 inflammasome (Shi et al., 2015) with the recruitment of apoptosis-associated Speck-like protein having a caspase-recruitment domain (ASC). Once activated, inflammasome can recruit pro-caspase-1 able to trigger both apoptosis (Dorweiler et al., 2014) and the activation of IL-1β/IL-18 inflammatory cytokines, which subsequently induce and "propagate" inflammatory responses. Furthermore, the inflammasome activation in macrophages and other cells may cause necroptosis (Karunakaran et al., 2016), an inflammatory type of cell death that eventually releases alarmins in the tissue and in the blood. This condition creates a microenvironment/niche which in turn produces cytokines and chemokines attracting others macrophages and lymphocytes. The inflammasome has also a key role in inflammaging as it triggers the NF-kB pathway representing one of the main hubs for inflammatory response in both immunosenescence and in the aging process (Franceschi et al., 2018b).

The main molecular patterns involved in the development of carotid plaque as well as in the aging process are illustrated in Fig. 2. Key molecular modulators crucial for apoptosis induction, DNA repair and autophagy processes (Stein and Matter, 2011; Swaminathan et al., 2014; Tabas, 2010), such as SIRT1, CHOP, OGG1, and MAP1LC3B, are highlighted, and the specific miRs capable of modulating their mRNA are reported in Supplementary Table 1S. Among those molecular modulators, SIRT1 is a well-known gene with impact in aging and longevity (Giuliani et al., 2018) and it has multiple functions. SIRT1 increases endothelial nitric oxide synthase–derived nitric oxide (Mattagajasingh et al., 2007) and has anti-inflammatory functions in endothelial cells and macrophages since downregulates the expression of various proinflammatory cytokines by interfering with the NF-κB signaling pathway (Chen et al., 2005). Gorenne et al. (2013) reported that reduced SIRT1 activity in VSMCs is associated with defective DNA repair, persistent DNA damage, DDR activation, reduced cell proliferation, premature senescence, and apoptosis, partly depending on the SIRT1 substrate NBS1. The same authors demonstrated that SIRT1 expression is reduced in human atherosclerosis both in plaques and in VSMCs cultured from human plaques.

The presence of unfolded/misplaced molecules may amplify the inflammatory response in a sort of vicious circle without any definitive resolution. In fact, cholesterol crystals and other damage-associated molecular patterns (DAMPs), such as HMGB1, HSPs, hyaluronans and S100 proteins, are present in the atherosclerotic lesion and may activate the inflammasome within macrophages, leading to the release of IL-1β, IL-18, and other pro-inflammatory cytokines (Duewell et al., 2010). These molecules are chemotactic for other inflammatory cells, including T cells and B cells which are additional crucial drivers of atherosclerosis (Warnatsch et al., 2015). The role of IL-1β appears to be particularly relevant inside the plaque niche (Grebe et al., 2018) while that of IL-18 at the site-specific carotid plaque and at systemic level is controversial (Kaplanski, 2018).

Inside the plaque, the presence of molecules which have the characteristics of molecular garbage ("garb-aging") (Franceschi et al., 2017) creates a persistent pro-inflammatory niche self-sustained by the activation of innate and adaptive immunity and may exacerbate the inflammatory response preventing the resolution of inflammation/inflammaging. The continuous recruitment of monocytes, foam cells and extracellular lipid accumulation eventually result in increased stenosis, unstable plaque development and risk of plaque rupture (Ait-Oufella et al., 2011; Moore et al., 2013) whose components/garbage can propagate the damage to the brain. Late plaque is characterized by a massive cell apoptosis/necroptosis due to inflammasome activation (Zhaolin et al., 2019) and accumulation of cells with senescent features.

Within the plaque niche, cell senescence can be favored by the increase of DNA damage (Martinet et al., 2002; Matturri et al., 2001; Shah et al., 2018) possibly occurring in all cell types present in the plaque, including macrophages, VSMCs, and endothelial cells. An *in vitro* study showed that plaque VSMCs undergo morphological features of senescence such as increased senescence-associated beta-galactosidase expression, reduced proliferation, and premature senescence. In addition, changes in cyclins D/E, p16, p21, and pRB are VSMC senescence mediators (Matthews et al., 2006), confirming the role of cell senescence in the plaque niche.

In turn, a DNA damage response (DDR) favors the cell senescence secretome-mediated pro-inflammatory microenvironment (Gardner et al., 2015), and the accumulation of senescent endothelial cells in the arteries of elderly persons likely induces a chronic systemic sterile inflammation and a vascular remodeling (systemic atherosclerotic disease) (Katsuumi et al., 2018).

Thus, two cellular processes, *i.e.* necroptosis and cell senescence, support a pro-inflammatory status and lead to the formation of a necrotic core that ultimately causes fragility and vulnerability of the plaque with high risk of plaque rupture and subsequent embolization or acute vascular occlusion. This extreme inflammatory status represents one of the highest risk conditions for the occurrence of cerebro-vascular events. (Pelisek et al., 2012, 2009).

Recently, the possible contribution of the microbiome in plaque formation (Jonsson and Bäckhed, 2017) has been suggested, adding another piece of evidence to the large literature on the role of microbiome on CVDs (Liberale et al., 2020). In particular, a pro-atherogenic effect of specific gut microbiota metabolites, such as γ-butyrobetaine and its down-stream products obtained from dietary carnitine, has been reported (Skagen et al., 2016; Wang et al., 2011). Overall, specific gut-microbiome derived metabolites, such as trimethylamine n-oxide and p-cresyl sulfate, can contribute to the plaque development and be considered significant predictors of plaque burden (*i.e.* total plaque area) (Bogiatzi et al., 2018). Furthermore, association of mycobiotic component with atherosclerosis (Chacón et al., 2018) and of bacterial species such as genus *Collinsella* (Karlsson et al., 2012) with symptomatic carotid plaque have been identified.

4. Epigenetic changes and microRNAs (miRs)

Recently, evidence of epigenetic changes associated with atherosclerosis emerged. In particular, important modifications in di-methylation patterns of histone H3 at different lysine positions have been identified in atherosclerotic lesions together with the decrease of DNA-methyltransferase DNMT1 and the increase of DNA-demethylase TET1 expression (Greißel et al., 2015). Additionally, changes have been identified in the expression of histone acetyltransferases, thus making histone acetylation and methylation crucial players in plaque development and stages. (Greißel et al., 2016).

In addition to these plaque-specific epigenetic changes it would be relevant from a diagnostic, prognostic and preventative point of view to identify circulating blood biomarkers able to characterize carotid plaque phenotypes (stable *vs* vulnerable). For example, a high level of plaque calcification is not necessarily predictive of lower vulnerability. In fact, cerebral ischemic lesions may be even more frequent in the presence of highly calcified plaques (Pini et al., 2017). These considerations are particularly important for asymptomatic patients.

In this regard, an impressive amount of data focused on various circulating blood biomarkers, such as inflammatory molecules/cytokines, metalloproteinases, lipoproteins, VSMC-related growth factors, and circulating endothelial progenitor cells (Chironi et al., 2007; Lau et al., 2007) have been found to be associated with carotid atherosclerosis (Martinez et al., 2020). All these markers may contribute to a general evaluation of the patients but they are not yet clinically relevant, since most of them are not carotid-specific and not able to properly identify asymptomatic patients at major risk to develop cerebrovascular events. Recently, tissue and circulating blood miRs attracted the attention of investigators in the field, given the widespread and pervasive effects of miRs both as modulators of gene expression and

biomarkers of health condition, as recently described (Dolz et al., 2017; Maitrias et al., 2017; Olivieri et al., 2017). In particular, miR-profiling could be performed on plaque surgical material and on blood, thus reflecting on one side the local carotid-specific alterations and on the other offering a tool to monitor patients at systemic level. The simultaneous evaluation of miRs in these two districts (plaque and blood) could give accurate information about a circulating miR-based signature and a specific plaque-phenotype. This task is challenging, and the available knowledge is summarized below. This relevant topic is here reviewed and up-graded in term of miR nomenclature (-3p or -5p, when possible).

First of all, miR-21-5p; miR-126-5p; miR-126-3p and miR-146a-5p, are potentially informative candidate being involved in the modulation of inflammation/inflammaging (inflamma-miRs) (Olivieri et al., 2013) and accordingly, some of those in the blood (or other body fluid) and in the plaque have been identified both in symptomatic and asymptomatic patients.

The Tampere vascular study (Raitoharju et al., 2011) compared miR-expression profiles in human aortic, carotid, and femoral atherosclerotic plaques, and control non-atherosclerotic internal thoracic arteries. Expression levels of miR-21-5p, miR-34a-5p, miR-146a-5p, miR-146b-5p, and miR-210-3p were significantly higher in atherosclerotic arteries than in control arteries. In addition, 187 predicted targets of the above mentioned five miRs were found to be downregulated in the carotid plaque, and most of these genes were involved in signal transduction, in transcription regulation and vesicular transport (Raitoharju et al., 2011).

Cipollone et al. (2011) reported that the expression levels of miR-100-5p, miR-127-5p, miR-127-3p, miR-133a-3p, miR-133b, and miR-145-5p were significantly higher in carotid plaques from symptomatic patients than asymptomatic patients who underwent endarterectomy. The same Authors have confirmed in *in vitro* experiments the effect of those miRs on stroke-related proteins, thus the potential therapeutic target of the same miRs has been highlighted.

Santovito et al. (2013) reported that miR-145-5p expression was higher in carotid plaques obtained from asymptomatic hypertensive patients with high-grade (> 70 %) carotid artery stenosis. Maitrias et al. (2015) reported that many other miRs (miR-100-5p, miR-125a-5p, miR-127-3p, miR-133a-3p, and miR-221-3p), including mir-145-5p were able to distinguish between symptomatic *versus* asymptomatic plaques and in particular miR-125a-5p expression was significantly and inversely correlated with the circulating level of LDL cholesterol. In a similar study, two different miRs, *i.e.* miR-21-5p and miR-143-3p, were found to be significantly upregulated in patients with asymptomatic plaques (Markus et al., 2016). Moreover, blood levels of miR-145-5p have been found significantly higher in patients with ischemic stroke than in control subjects (Gan et al., 2012; Xu et al., 2018). Another study by Zhang et al. (2016) found that serum miR-320b expression is closely related to plaque stability, but not with plaque diameter, and is downregulated in patients with vulnerable plaque.

Huang et al. (2017) found that plasma miR-92a-3p was increased in hypertensive patients and was correlated with carotid intima-media thickness, in comparison with healthy controls. MiR-92a-3p levels were also positively associated with other related parameters such as carotid–femoral pulse wave velocity and ambulatory blood pressure. These findings suggest that miR-92a-3p may be involved in the pathophysiology of hypertension and atherosclerosis, and might be a predictor of atherosclerosis in patients with hypertension. In a recent study, patients with acute stroke and carotid stenosis were found to have decreased miR-181b-5p serum levels in comparison with the control group. Furthermore, miR-181b-5p could attenuate plaque vulnerability by modulating the polarization of macrophage towards M2 macrophages (An et al., 2017).

Badacz et al. (2018) found that serum miR-1-3p and miR-133b were increased in symptomatic patients as compared to asymptomatic. Authors showed that the miRs levels differ significantly with respect to carotid plaque morphology and could be useful in monitoring or predicting future cerebral ischemic event.Other circulating miRs, such as miR-21-5p and miR-221-3p were shown to be higher in patients with stroke and carotid atherosclerosis than in healthy controls, while in a population of T2D patients, urine levels of miR-29b-3p were shown to be significantly correlated with carotid intima-media thickness. Thus, the presence of morbidities or comorbidities reveals different miR profiles, and miR-21-5p, miR-221-3p and miR-29b-3p likely modulate carotid atherosclerosis in pathological conditions (Peng et al., 2013; Tsai et al., 2013). Bazan et al. (2015) showed a decrease of miR-221-3p and miR-222-3p in symptomatic carotid plaque with an increase in its target, p27 and in a subsequent study, serum miR-221-3p was found lower in the symptomatic cohort. Furthermore, the same authors have identified the role of a non-coding RNA, circular RNA (circR) in predicting plaque rupture. In particular, the circR-284/miR-221-3p ratio was found increased in symptomatic patients' serum, and validated in group with severe and urgent conditions only (Bazan et al., 2017). Another potential biomarker could be the circulating miR-638, found associated with atherosclerotic plaque vulnerability in patients with high-grade carotid stenosis (Luque et al., 2018).

New researches have highlighted the possible role of miR-210-3p as modulator of plaque stability in carotid atheroma being decreased in vulnerable carotid plaque. This finding was also indirectly confirmed by miR-210-3p low levels in the local plasma collected from the lesion site (Eken et al., 2017).

Furthermore, we have recently shown the possibility to distinguish subtypes of calcification in carotid plaques by different miR-signatures where miR-30a-5p and miR-30d directly correlated with calcification extension and thickness at angio- computed tomography imaging (Vasuri et al., 2019).

Magenta et al. (2018) analyzed carotid plaque instability in patients undergoing endarterectomy by correlating the miR-200c-3p expression with different markers. Higher expression of miR-200c-3p positively correlated with several inflammatory markers such as monocyte chemoattractant protein-1, cyclooxygenase-2, IL-6, MMPs, and miRs-33a/b-5p, and negatively correlated with other markers, such as ZEB1 and SIRT1. Among those, miRs-33a/b-5p have been associated with carotid plaque progression (Karunakaran et al., 2015) and are the most prominent miRs involved in cholesterol homeostasis (Rayner et al., 2010). MiR-200c-3p may be regulated by miRs-33a/b-5p *via* targeting of ZEB1, as observed in a familial hypercholesterolemia model (D'Agostino et al., 2017). MiR-200c-3p was also increased in patients' plasma before endarterectomy, thus it could be clinically useful for identifying patients at high embolic risk, as suggested by the authors (Magenta et al., 2018).

Overall, the current literature shows a complex framework where many variables, such as presence of different patient cohorts, morbidities/comorbidities, and other variables not specifically considered here but present in the above-mentioned papers (drug-therapies, gender, body mass index, age and control tissues, technology mirRs detection) make difficult to identify a single/clear circulating miR-signatures associated with carotid plaque phenotypes. Table 2 shows the most significant up-or down-regulated miRs in different comparisons. Importantly, miR-21-5p; miR-100-5p; -127-3p; -133a/b-3p and -221-3p appear overrepresented suggesting their confirmed role in different studies both as epigenetic modulators inside the plaque and in some cases, as blood circulating markers.

Additional miRs, *i.e.* miR-1-3p; -19b-3p; -21 (-3p or-5p); -22-3p; -24-3p; -27b-3p; -34a-5p; -145-5p; -181a/b-5p; -200c-3p and -320b, are present in both Table 2 and Table 1S, thus their respective potential targets involved in ER stress/autophagy/apoptosis regulation emerge in Table 1S. Among those targets, SIRT1 potentially undergoes regulation by several miRs, *i.e.* miR-1-3p; -22-3p; -27b-3p; -181a/b-5p; -200c-3p and -320b, thus apparently subjected to a fine tune in comparison with the others.

5. Anti-plaque prevention and therapies

Prevention of carotid atherosclerosis follows the general guidelines, *i.e.* recommendation for a healthy life style, such as daily personalized physical activity and a well-balanced diet such as Mediterranean diet (Gardener et al., 2014; Lakkur and Judd, 2015), avoiding tobacco smoking, alcohol consumption, among others. In particular, the effect of smoking has been investigated in epigenetic changes of carotid and plaque development/progression (Siemelink Marten et al., 2018). Data indicate that carotid endarterectomy is proposed to be required on average 7 years earlier in smokers (Redgrave et al., 2010).

The molecular mechanisms highlighted in Fig. 2 suggest that basic phenomena shared with the aging process converge in creating a self-sustained inflamed microenvironment or niche. Specific molecules, such as resolvines/maresins/protectines capable of counteracting and limiting plaque formation, could be crucial and promising anti-plaque therapies (Akagi et al., 2015; Fredman et al., 2016; Rea et al., 2018), but scanty data on this topic are available in humans.

The same considerations regard anti-senolytic approaches, where only data on animal models are available. Intermittent treatment with Dasatinib and Quercetin (*via* oral gavage), aiming at the pharmacological clearance of senescent cells, alleviates vasomotor dysfunction in naturally aging mice and mice with established atherosclerosis. Senescent cell clearance reduces the markers of osteogenesis in advanced intimal plaques, finally reducing calcification (Roos et al., 2016). Zhu et al. (2015) demonstrated that cardiac function and carotid vascular reactivity were improved five days after a single dose of Dasatinib and Quercetin in old mice.

The potential benefit of anti-senolytic drugs in humans is not yet clarified and precautions should be envisaged owing the above-mentioned cellular and molecular complexity of the atherosclerotic niche and its development. Thus, it is reasonable to predict that senescent cell clearance will be an effective complementary therapy to classical drugs (anti-inflammatory/statins) to reduce morbidity and mortality associated with age-related CVDs, and many studies in this direction are expected in the next years.

Thus, the aim of the current therapeutic approach is to decelerate the local progression of the plaque and the level of systemic inflammation, *i.e.* antiplatelet agents, statins, and angiotensin-converting enzyme inhibitors, as recently reviewed (Donato et al., 2018; Naylor and Ricco, 2018). Data obtained in rats suggest an important effect of long-term pharmacological inhibition of angiotensin II type 1 receptor with a specific antagonist inhibitor. This treatment results in the doubling of lifespan in hypertensive animals, together with improvement in cardiac function and metabolism, and enhanced endothelial function (Linz et al., 2000), suggesting a pervasive effect of the anti- hypertensive therapy at the carotid-site as well at the systemic levels (Alfaras et al., 2016).

Since vulnerable plaques show evidence of VSMCs death, the low number of VSMCs in the fibrous cap may suggest the need of treatments in order to stabilize existing plaque by targeting VSMCs. In this perspective, the upregulated miR-21-5p expression has been shown to inhibit reactive oxygen species–induced apoptosis and death in VSMCs (Lin et al., 2009). In mice, miR-126-3p–treated arteries displayed higher intimal smooth muscle cell counts, a higher collagen content, and fewer apoptotic cells (compared to controls). These changes are consistent with a greater plaque stability (Zernecke et al., 2009). Thus, promoting a contractile VSMC phenotype may increase the integrity of the fibrous cap, and these findings pave the way for a site-specific treatment of plaque.

On the other hand, trans-differentiation of VSMCs from the normal, quiescent, and contractile, phenotype to the synthetic, proliferative phenotype is associated with atherosclerosis. Interestingly, it has been reported that several miRs, including miR-126-3p, are involved in this process (Metzinger-Le Meuth et al., 2017), thus making miRs good targets for such a therapeutical approach. In this setting, lentiviral delivery of miR-145 in smooth muscle cell in ApoE−/− mice have reduced plaque volume and increased features of plaque stability (such as a higher collagen content and a greater fibrous cap surface area) in a manner consistent with the promotion of a quiescent smooth muscle cell phenotype (Lovren et al., 2012), but further studies need to confirm the results and applicability in humans.

MiRs regulating cholesterol homeostasis have also been explored in animal models for their therapeutic potential in promoting the regression of atherosclerotic plaques. Several miRs were identified as effective modulators of key genes in lipoprotein metabolism (Laffont and Rayner, 2017), such as LDL-receptor (LDLR) and ATP-binding cassette A1 (ABCA1). In addition, a large amount of miRs, including miR-26a-5p; -33a/b-5p; -106a-5p;-144-3p; -128-3p; -130b-3p; -148a-3p; -301b-3p; -302a-3p and -758-3p, are able to promote foam cell formation by inhibiting macrophage cholesterol efflux *via* ABCA1 (Feinberg and Moore, 2016; Goedeke et al., 2015). Among miRs targeting ABCA1, miRs-33a/b-5p have a significant impact, potentially influencing the growth of atherosclerotic plaque, regulating autophagy, cellular phenotype and macrophage metabolism (Yao et al., 2018; Feinberg and Moore, 2016; Ouimet et al., 2015). MiRs-33a/b-5p are able to down-regulate key effectors and transcriptional activators including FOXO3 and TFEB, leading to a reduced phagocytosis and lysosomal activity within macrophages (Yao et al., 2018). Accordingly, the inhibition of miRs-33a/b-5p expression in *in vivo* mice model resulted in an increase of the hepatic ABCA1 expression and in plasma HDL levels facilitating atherosclerosis regression, thus confirming the role of miRs-33a/b-5p on the regulation of lipid metabolism (Churov et al., 2019; Najafi-Shoushtari et al., 2010). Overall, the use of carotid-specific biomarkers or circulating miRs associated to carotid plaque stability could be essential for patient monitoring, especially if biomarkers will be able to predict plaque rupture.

Currently, the therapeutic management of carotid plaques strongly relies on the severity of stenosis as the primary guide to choose the most appropriate intervention. The current clinical European guidelines (Naylor and Ricco, 2018) recommend surgical endarterectomy for: i) symptomatic patients with carotid territory symptoms within the preceding six months caused by > 50 % stenosis of the carotid artery; ii) asymptomatic patients with > 70 % stenosis of the carotid artery.

6. Conclusions

Overall, carotid plaque lesion can be considered a tissue site-specific case of accelerated aging, *i.e.* a sort of chronic pro-inflammatory macroniche. As described above, the molecular and cellular events occurring within the niche include the classical plethora of basic aging mechanisms, such as mitochondria dysfunction, defective autophagy, ER stress, defective ubiquitin/proteasome system, inflammasome activation, DNA damage response, and cell senescence with its characteristic senescence associated secretory phenotype. Table 1 summarizes the main studies performed in the framework of human carotid plaque highlighiting the seven pillars identified by Geroscience, and involved in the age-related diseases (Franceschi et al., 2018a).

These mechanisms are also found in those pathologies where unhealthy life-style and lifelong exposure of noxious agents/bacteria/viruses may accelerate end-stage organ diseases, such as liver and kidney diseases among others (Kooman et al., 2017; Morsiani et al., 2019). Thus, a tight relationship appears to exist between carotid plaque/atherosclerosis development and exposure to a variety of external and internal agents (“exposomes”) (Wild, 2012), such as life style, environment, socioeconomic status and diet, among others. For example, a recent study in mice suggests an effect of red meat consumption, able to favor the induction of “xeno-autoantigen” in humans (*via* metabolic incorporation of carbohydrate residues into endogenous glycoconjugates) and the increase of circulating anti-“xeno-auto-antibodies” driving chronic inflammation and favoring/accelerating plaque formation (Kawanishi et al., 2019).

Table 1
The seven pillars of aging and the related mechanisms involved in human carotid plaque.

The seven pillars of aging in the framework of carotid atheroma	References
Metabolism	
Altered metabolites	Tomas et al., 2018; Tzoulaki et al., 2019.
Gut microbiota	Karlsson et al., 2012; Skagen et al., 2016; Bogiatzi et al., 2018.
Inflammation	
Inflammatory molecules	Ross, 1999; Schillinger et al., 2005; Ammirati et al., 2015.
Activation of inflammasome	Shi et al., 2015.
Epigenetics	
Histone and chromatin modification	Greißel et al., 2015, 2016.
MicroRNAs	Raitoharju et al., 2011; Cipollone et al., 2011; Santovito et al., 2013; Tsai et al., 2013; Maitrias et al., 2015; Bazan et al., 2015; Zhang et al., 2016; Markus et al., 2016; An et al., 2017; Eken et al. 2017; Magenta et al., 2018; Badacz et al., 2018; Luque et al., 2018.
Macromolecular damage (Cellular senescence)	
Cellular senescence	Gardner et al., 2015; Katsuumi et al., 2018.
DNA damage	Matturri et al., 2001; Martinet et al., 2002; Matthews et al., 2006; Yuan et al., 2010; Gorenne et al., 2013; Shah et al., 2018
Stem cells and regeneration	
Regulation of stem cell functions, proliferation and apoptosis	Chironi et al., 2007; Lau et al., 2007.
Proteostasis	
Defective ubiquitin/proteasome system (misfolded/ oxidized proteins)	Versari et al., 2006; Marfella et al., 2006; Herrmann et al., 2008; Dorweiler et al., 2014.
Adaptation to stress	
Mitochondrial dysfunction	Docherty et al., 2018; Vendrov et al., 2015.
Endoplasmic Reticulum stress	Tabas, 2010; Garbin et al., 2014.
Defective autophagy	Swaminathan et al., 2014; Liu et al., 2015; Alloza et al., 2016.

Table 2
Up-regulated or down-regulated miRs in different comparisons and tissues.

hsa-miRs	Patients/Plaque comparison	Tissue	Expression Change	References
miR-21-5p	Carotid endarterectomy *vs* Control	Carotid plaques	↑	Raitoharju et al. (2011)
miR-34a-5p			↑	
miR-210-3p			↑	
miR-100-5p	Unstable Symptomatic *vs* Asymptomatic	Carotid plaques	↑	Cipollone et al. (2011)
miR-127			↑	
miR-133a-3p			↑	
miR-133b			↑	
miR-145 − 5p			↑	
miR-145-5p	Stable asymptomatic Hypertensive *vs* Stable asymptomatic non-hypertensive	Carotid plaques	↑	Santovito et al. (2013)
miR-100-5p	Unstable symptomatic	Carotid plaques	↑	Maitrias et al. (2015)
miR-125a-5p	*vs* Asymptomatic		↑	
miR-127-3p			↑	
miR-133a-3p			↑	
mir-145-5p			↑	
miR-221-3p			↑	
miR-221-3p	Acutely unstable symptomatic *vs* Asymptomatic	Carotid plaques	↓	Bazan et al. (2015)
miR-222-3p			↓	
miR-21-5p miR-143-3p	Stable asymptomatic *vs* Symptomatic; Asymptomatic *vs* Control	Carotid plaques	↑	Markus et al. (2016)
miR-19b-3p			↑	
miR-22-3p			↑	
			↑	
miR-320b	Unstable Symptomatic *vs* Asymptomatic	Serum	↓	Zhang et al. (2016)
miR-181b-5p	Unstable Symptomatic *vs* Control	Serum	↓	An et al., (2017)
miR-221-3p	Acutely unstable symptomatic *vs* Asymptomatic	Serum	↓	Bazan et al. (2017)
miR-199b-3p	Stable asymptomatic with stenosis progression *vs* Asymptomatic	Peripheral blood exosomes	↑	Dolz et al. (2017)
miR-27b-3p	without		↑	
miR-130a-3p			↑	
miR-221-3p			↑	
miR-24-3p			↑	
miR-210-3p	Unstable Symptomatic *vs* Asymptomatic	Carotid plaques and plasma within	↓	Eken et al., (2017)
miR-200c-3p	Unstable Symptomatic *vs* Asymptomatic	Carotid plaques/Serum	↑	Magenta et al. (2018)
miR-1-3p	Unstable Symptomatic *vs* Asymptomatic	Serum	↑	Badacz et al. (2018)
miR-133b			↑	
miR-638	Unstable Symptomatic *vs* Control group	Serum	↓	Luque et al. (2018)

Within the Geroscience perspective, a main question is the feasibility of combating aging, halting atherosclerosis and plaque formation. The deceleration of the aging process is apparently possible by optimizing the life style (calorie restriction, Mediterranean diet, avoiding smoking and alcohol abuse, personalized daily physical exercise), thus decreasing inflammaging and cardiometabolic risk (Campisi et al., 2019; Kraus et al., 2019). In this regard, long lived subjects and centenarians represent "a successful slowed aging" (regular food timing and small portions) (Franceschi et al., 2018c), and it has been shown that the prevalence of carotid plaques decreases with increasing paternal age/longevity (Zureik et al., 2006). In particular, plaque occurrence has been found decreased after the tenth decade of life (Homma et al., 2001). A relatively low plaque frequency in centenarians suggests that at least some centenarians may possess unknown genetic or acquired characteristics conferring resistance or resilience to plaque progression. It is interesting to note that centenarians have a decreased level of circulating miR-21-5p in the blood (Olivieri et al., 2012), and this could be a piece of the puzzle to explain genetic/epigenetic differences able to accelerate or decelerate aging/plaque formation.

In the current review, we did not address the genetic risk profile of CVDs and atherosclerosis, which is determinant for the human longevity (Franceschi et al., 2020) but outside of the scope of this review. However, a 15 % of CVDs events are not associated with the abovementioned common risk factors, and in these cases the genetic variants could play a major role (Fig. 1).

In this complex scenario, patients with carotid stenosis may be evaluated for several variables such as, early or not patients, socioeconomic status, type of plaque (stable *vs* vulnerable) and for different risk factors that may accelerate plaque instability and stroke risk. Thus, the biological age of arteries and not the chronological age of patients (Chan et al., 2019; Hamczyk et al., 2020; Li et al., 2020; Moskalev, 2020; Pyrkov and Fedichev, 2019), becomes the relevant parameter for carotid plaque assessment, prognosis and risk prediction. Further studies need to identify the proper circulating molecular signature (likely based on miRs) in the different health conditions for the personalized/precise evaluation of atherosclerosis level, plaque formation and biological age of arteries, thus going in deeper than previously done.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.arr.2020.101090.

References

Ait-Oufella, H., Taleb, S., Mallat, Z., Tedgui, A., 2011. Recent advances on the role of cytokines in atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 31, 969–979. https://doi.org/10.1161/ATVBAHA.110.207415.

Akagi, D., Chen, M., Toy, R., Chatterjee, A., Conte, M.S., 2015. Systemic delivery of proresolving lipid mediators resolvin D2 and maresin 1 attenuates intimal hyperplasia in mice. FASEB J. 29, 2504–2513. https://doi.org/10.1096/fj.14-265363.

Alfaras, I., Di Germanio, C., Bernier, M., Csiszar, A., Ungvari, Z., Lakatta, E.G., de Cabo, R., 2016. Pharmacological strategies to retard cardiovascular aging. Circ. Res. 118, 1626–1642. https://doi.org/10.1161/CIRCRESAHA.116.307475.

Alloza, I., Goikuria, H., Freijo, M., del, M., Vandenbroeck, K., 2016. A role for autophagy in carotid atherosclerosis. Eur. Stroke J. 1, 255–263. https://doi.org/10.1177/2396987316674085.

Ammirati, E., Moroni, F., Norata, G.D., Magnoni, M., Camici, P.G., 2015. Markers of inflammation associated with plaque progression and instability in patients with carotid atherosclerosis. Mediators Inflamm. 2015, 718329. https://doi.org/10.1155/2015/718329.

An, T.-H., He, Q.-W., Xia, Y.-P., Chen, S.-C., Baral, S., Mao, L., Jin, H.-J., Li, Y.-N., Wang, M.-D., Chen, J.-G., Zhu, L.-Q., Hu, B., 2017. MiR-181b antagonizes atherosclerotic plaque vulnerability through modulating macrophage polarization by directly targeting Notch1. Mol. Neurobiol. 54, 6329–6341. https://doi.org/10.1007/s12035-016-0163-1.

Badacz, R., Przewłocki, T., Gacoń, J., Stępień, E., Enguita, F.J., Karch, I., Żmudka, K., Kabłak-Ziembicka, A., 2018. Circulating miRNA levels differ with respect to carotid plaque characteristics and symptom occurrence in patients with carotid artery stenosis and provide information on future cardiovascular events. Postepy Kardiol. Interwencyjnej 14, 75–84. https://doi.org/10.5114/aic.2018.74358.

Bazan, H.A., Hatfield, S.A., O'Malley, C.B., Brooks, A.J., Lightell, D., Woods, T.C., 2015. Acute loss of miR-221 and miR-222 in the atherosclerotic plaque shoulder accompanies plaque rupture. Stroke 46, 3285–3287. https://doi.org/10.1161/STROKEAHA.115.010567.

Bazan, H.A., Hatfield, S.A., Brug, A., Brooks, A.J., Lightell, D.J., Woods, T.C., 2017. Carotid plaque rupture is accompanied by an increase in the ratio of serum circR-284 to miR-221 levels. Circ. Cardiovasc. Genet. 10. https://doi.org/10.1161/CIRCGENETICS.117.001720.

Bentzon, J.F., Otsuka, F., Virmani, R., Falk, E., 2014. Mechanisms of plaque formation and rupture. Circ. Res. 114, 1852–1866. https://doi.org/10.1161/CIRCRESAHA.114.302721.

Bogiatzi, C., Gloor, G., Allen-Vercoe, E., Reid, G., Wong, R.G., Urquhart, B.L., Dinculescu, V., Ruetz, K.N., Velenosi, T.J., Pignanelli, M., Spence, J.D., 2018. Metabolic products of the intestinal microbiome and extremes of atherosclerosis. Atherosclerosis 273, 91–97. https://doi.org/10.1016/j.atherosclerosis.2018.04.015.

Bots, M.L., Breslau, P.J., Briët, E., de Bruyn, A.M., van Vliet, H.H., van den Ouweland, F.A., de Jong, P.T., Hofman, A., Grobbee, D.E., 1992. Cardiovascular determinants of carotid artery disease. Rotterdam Elderly Study. Hypertension 19, 717–720.

Campisi, J., Kapahi, P., Lithgow, G.J., Melov, S., Newman, J.C., Verdin, E., 2019. From discoveries in ageing research to therapeutics for healthy ageing. Nature 571, 183. https://doi.org/10.1038/s41586-019-1365-2.

Capri, M., Franceschi, C., Salvioli, S., 2020. Inflammaging targets. In: Rattan, S.I.S. (Ed.), Encyclopedia of Biomedical Gerontology. Academic Press, Oxford, pp. 271–278. https://doi.org/10.1016/B978-0-12-801238-3.11283-8.

Cevenini, E., Invidia, L., Lescai, F., Salvioli, S., Tieri, P., Castellani, G., Franceschi, C., 2008. Human models of aging and longevity. Expert Opin. Biol. Ther. 8, 1393–1405. https://doi.org/10.1517/14712598.8.9.1393.

Chacón, M.R., Lozano-Bartolomé, J., Portero-Otín, M., Rodríguez, M.M., Xifra, G., Puig, J., Blasco, G., Ricart, W., Chaves, F.J., Fernández-Real, J.M., 2018. The gut mycobiome composition is linked to carotid atherosclerosis. Benef. Microbes 9, 185–198. https://doi.org/10.3920/BM2017.0029.

Chan, M.S., Arnold, M., Offer, A., Hammami, I., Mafham, M., Armitage, J., Perera, R., Parish, S., 2019. Biological age in UK Biobank: biomarker composition and prediction of mortality, coronary heart disease and hospital admissions. medRxiv 2019https://doi.org/10.1101/2019.12.12.19014720. 12.12.19014720.

Chen, L.-F., Williams, S.A., Mu, Y., Nakano, H., Duerr, J.M., Buckbinder, L., Greene, W.C., 2005. NF-κB RelA phosphorylation regulates RelA acetylation. Mol. Cell. Biol. 25, 7966–7975. https://doi.org/10.1128/MCB.25.18.7966-7975.2005.

Chironi, G., Walch, L., Pernollet, M.-G., Gariepy, J., Levenson, J., Rendu, F., Simon, A., 2007. Decreased number of circulating CD34 + KDR + cells in asymptomatic subjects with preclinical atherosclerosis. Atherosclerosis 191, 115–120. https://doi.org/10.1016/j.atherosclerosis.2006.02.041.

Churov, A., Summerhill, V., Grechko, A., Orekhov, A.V., Orekhov, A., 2019. MicroRNAs as potential biomarkers in atherosclerosis. Int. J. Mol. Sci. 20, 5547. https://doi.org/10.3390/ijms20225547.

Cipollone, F., Felicioni, L., Sarzani, R., Ucchino, S., Spigonardo, F., Mandolini, C., Malatesta, S., Bucci, M., Mammarella, C., Santovito, D., Lutiis, Fde, Marchetti, A., Mezzetti, A., Buttitta, F., 2011. A unique MicroRNA signature associated with plaque instability in humans. Stroke 42, 2556–2563. https://doi.org/10.1161/STROKEAHA.110.597575.

D'Agostino, M., Martino, F., Sileno, S., Barillà, F., Beji, S., Marchetti, L., Gangi, F.M., Persico, L., Picozza, M., Montali, A., Martino, E., Zanoni, C., Avitabile, D., Parrotto, S., Capogrossi, M.C., Magenta, A., 2017. Circulating miR-200c is up-regulated in paediatric patients with familial hypercholesterolaemia and correlates with miR-33a/b levels: implication of a ZEB1-dependent mechanism. Clinical science (London, England: 1979) 131, 2397–2408. https://doi.org/10.1042/CS20171121.

de Weerd, M., Greving, J.P., Hedblad, B., Lorenz, M.W., Mathiesen, E.B., O'Leary, D.H., Rosvall, M., Sitzer, M., de Borst, G.J., Buskens, E., Bots, M.L., 2014. Prediction of asymptomatic carotid artery stenosis in the general population: identification of high-risk groups. Stroke 45, 2366–2371. https://doi.org/10.1161/STROKEAHA.114.005145.

Docherty, C.K., Carswell, A., Friel, E., Mercer, J.R., 2018. Impaired mitochondrial respiration in human carotid plaque atherosclerosis: a potential role for Pink1 in vascular smooth muscle cell energetics. Atherosclerosis 268, 1–11. https://doi.org/10.1016/j.atherosclerosis.2017.11.009.

Dolz, S., Górriz, D., Tembl, J.I., Sánchez, D., Fortea, G., Parkhutik, V., Lago, A., 2017. Circulating MicroRNAs as novel biomarkers of stenosis progression in asymptomatic carotid stenosis. Stroke 48, 10–16. https://doi.org/10.1161/STROKEAHA.116.013650.

Donato, A.J., Machin, D.R., Lesniewski, L.A., 2018. Mechanisms of dysfunction in the aging vasculature and role in age-related disease. Circ. Res. 123, 825–848. https://doi.org/10.1161/CIRCRESAHA.118.312563.

Dorweiler, B., Grechowa, I., Wallrath, A., Vahl, C.F., Horke, S., 2014. Activation of the proapoptotic unfolded protein response in plaques of the human carotid artery. Eur. J. Vasc. Endovasc. Surg. 48, 248–257. https://doi.org/10.1016/j.ejvs.2014.06.038.

Duewell, P., Kono, H., Rayner, K.J., Sirois, C.M., Vladimer, G., Bauernfeind, F.G., Abela, G.S., Franchi, L., Nuñez, G., Schnurr, M., Espevik, T., Lien, E., Fitzgerald, K.A., Rock, K.L., Moore, K.J., Wright, S.D., Hornung, V., Latz, E., 2010. NLRP3 inflamasomes are required for atherogenesis and activated by cholesterol crystals that form early in disease. Nature 464, 1357–1361. https://doi.org/10.1038/nature08938.

Feinberg, M.W., Moore, K.J., 2016. MicroRNA regulation of atherosclerosis. Circ. Res. 118, 703–720. https://doi.org/10.1161/CIRCRESAHA.115.306300.

Ferrucci, L., Fabbri, E., 2018. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat. Rev. Cardiol. 15, 505–522. https://doi.org/10.1038/s41569-018-0064-2.

Flaherty, M.L., Kissela, B., Khoury, J.C., Alwell, K., Moomaw, C.J., Woo, D., Khatri, P., Ferioli, S., Adeoye, O., Broderick, J.P., Kleindorfer, D., 2013. Carotid artery stenosis as a cause of stroke. Neuroepidemiology 40, 36–41. https://doi.org/10.1159/000341410.

Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann. N. Y. Acad. Sci. 908, 244–254.

Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., Panourgia, M.P., Invidia, L., Celani, L., Scurti, M., Cevenini, E., Castellani, G.C., Salvioli, S., 2007. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech. Ageing Dev. 128, 92–105. https://doi.org/10.1016/j.mad.2006.11.016.

Franceschi, C., Garagnani, P., Vitale, G., Capri, M., Salvioli, S., 2017. Inflammaging and 'Garb-aging.'. Trends Endocrinol. Metab. 28, 199–212. https://doi.org/10.1016/j.tem.2016.09.005.

Franceschi, C., Garagnani, P., Morsiani, C., Conte, M., Santoro, A., Grignolio, A., Monti, D., Capri, M., Salvioli, S., 2018a. The continuum of aging and age-related diseases: common mechanisms but different rates. Front. Med. (Lausanne) 5, 61. https://doi.org/10.3389/fmed.2018.00061.

Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., Santoro, A., 2018b. Inflammaging: a new immune–metabolic viewpoint for age-related diseases. Nat. Rev. Endocrinol. 14, 576. https://doi.org/10.1038/s41574-018-0059-4.

Franceschi, C., Ostan, R., Santoro, A., 2018c. Nutrition and Inflammation: Are Centenarians Similar to Individuals on Calorie-Restricted Diets? Annu. Rev. Nutr. 38, 329–356. https://doi.org/10.1146/annurev-nutr-082117-051637.

Franceschi, C., Garagnani, P., Olivieri, F., Salvioli, S., Giuliani, C., 2020. The contextualized genetics of human longevity: JACC focus seminar. J. Am. Coll. Cardiol. 75, 968–979.

Fredman, G., Hellmann, J., Proto, J.D., Kuriakose, G., Colas, R.A., Dorweiler, B., Connolly, E.S., Solomon, R., Jones, D.M., Heyer, E.J., Spite, M., Tabas, I., 2016. An imbalance between specialized pro-resolving lipid mediators and pro-inflammatory leukotrienes promotes instability of atherosclerotic plaques. Nat. Commun. 7, 12859. https://doi.org/10.1038/ncomms12859.

Gan, C.S., Wang, C.W., Tan, K.S., 2012. Circulatory microRNA-145 expression is increased in cerebral ischemia. Genet. Mol. Res. 11, 147–152. https://doi.org/10.4238/2012.January.27.1.

Garbin, U., Stranieri, C., Pasini, A., Baggio, E., Lipari, G., Solani, E., Mozzini, C., Vallerio, P., Cominacini, L., Fratta Pasini, A.M., 2014. Do oxidized polyunsaturated Fatty acids affect endoplasmic reticulum stress-induced apoptosis in human carotid plaques? Antioxid. Redox Signal. 21, 850–858. https://doi.org/10.1089/ars.2014.5870.

Gardener, H., Wright, C.B., Cabral, D., Scarmeas, N., Gu, Y., Cheung, K., Elkind, M.S.V., Sacco, R.L., Rundek, T., 2014. Mediterranean diet and carotid atherosclerosis in the Northern Manhattan Study. Atherosclerosis 234, 303–310. https://doi.org/10.1016/j.atherosclerosis.2014.03.011.

Gardner, S.E., Humphry, M., Bennett, M.R., Clarke, M.C.H., 2015. Senescent vascular smooth muscle cells drive inflammation through an Interleukin-1α-Dependent senescence-associated secretory phenotype. Arterioscler. Thromb. Vasc. Biol. 35, 1963–1974. https://doi.org/10.1161/ATVBAHA.115.305896.

Gebreab, S.Y., Diez-Roux, A.V., Hickson, D.A., Boykin, S., Sims, M., Sarpong, D.F., Taylor, H.A., Wyatt, S.B., 2012. The contribution of stress to the social patterning of clinical and subclinical CVD risk factors in african americans: the jackson heart study. Soc Sci Med 75, 1697–1707. https://doi.org/10.1016/j.socscimed.2012.06.003.

Giuliani, Cristina, Garagnani, Paolo, Franceschi, Claudio, 2018. Genetics of human longevity within an eco-evolutionary nature-nurture framework. Circ. Res. 123, 745–772. https://doi.org/10.1161/CIRCRESAHA.118.312562.

Goedeke, L., Rotllan, N., Canfrán-Duque, A., Aranda, J.F., Ramírez, C.M., Araldi, E., Lin, C.-S., Anderson, N.N., Wagschal, A., de Cabo, R., Horton, J.D., Lasunción, M.A., Näär, A.M., Suárez, Y., Fernández-Hernando, C., 2015. MicroRNA-148a regulates LDL receptor and ABCA1 expression to control circulating lipoprotein levels. Nat. Med. 21, 1280–1289. https://doi.org/10.1038/nm.3949.

Gorenne, Isabelle, Sheetal, Kumar, Kelly, Gray, Nichola, Figg, Haixiang, Yu, John, Mercer, Martin, Bennett, 2013. Vascular smooth muscle cell sirtuin 1 protects against DNA damage and inhibits atherosclerosis. Circulation 127, 386–396. https://doi.org/10.1161/CIRCULATIONAHA.112.124404.

Grebe, Alena, Florian, Hoss, Eicke, Latz, 2018. NLRP3 inflammasome and the IL-1 pathway in atherosclerosis. Circ. Res. 122, 1722–1740. https://doi.org/10.1161/CIRCRESAHA.118.311362.

Greißel, A., Culmes, M., Napieralski, R., Wagner, E., Gebhard, H., Schmitt, M., Zimmermann, A., Eckstein, H.-H., Zernecke, A., Pelisek, J., 2015. Alternation of histone and DNA methylation in human atherosclerotic carotid plaques. Thromb. Haemost. 114, 390–402. https://doi.org/10.1160/TH14-10-0852.

Greißel, A., Culmes, M., Burgkart, R., Zimmermann, A., Eckstein, H.-H., Zernecke, A., Pelisek, J., 2016. Histone acetylation and methylation significantly change with severity of atherosclerosis in human carotid plaques. Cardiovasc. Pathol. 25, 79–86. https://doi.org/10.1016/j.carpath.2015.11.001.

Hamczyk, M.R., Nevado, R.M., Barettino, A., Fuster, V., Andrés, V., 2020. Biological versus chronological aging: JACC focus seminar. J. Am. Coll. Cardiol. 75, 919–930. https://doi.org/10.1016/j.jacc.2019.11.062.

Herder, M., Johnsen, S.H., Arntzen, K.A., Mathiesen, E.B., 2012. Risk factors for progression of carotid intima-media thickness and total plaque area: a 13-year follow-up study: the Tromsø Study. Stroke 43, 1818–1823. https://doi.org/10.1161/STROKEAHA.111.646596.

Hermus, L., Lefrandt, J.D., Tio, R.A., Breek, J.-C., Zeebregts, C.J., 2010. Carotid plaque formation and serum biomarkers. Atherosclerosis 213, 21–29. https://doi.org/10.1016/j.atherosclerosis.2010.05.013.

Herrmann, J., Soares, S.M., Lerman, L.O., Lerman, A., 2008. Potential role of the ubiquitin-proteasome system in atherosclerosis: aspects of a protein quality disease. J. Am. Coll. Cardiol. 51, 2003–2010. https://doi.org/10.1016/j.jacc.2008.02.047.

Homma, S., Hirose, N., Ishida, H., Ishii, T., Araki, G., 2001. Carotid plaque and intima-media thickness assessed by b-mode ultrasonography in subjects ranging from young adults to centenarians. Stroke 32, 830–835. https://doi.org/10.1161/01.str.32.4.830.

Huang, Y., Tang, S., Ji-yan, C., Huang, C., Li, J., Cai, A.-p, Feng, Y.-q, 2017. Circulating miR-92a expression level in patients with essential hypertension: a potential marker of atherosclerosis. J. Hum. Hypertens. 31, 200–205. https://doi.org/10.1038/jhh.2016.66.

Jonsson, A.L., Bäckhed, F., 2017. Role of gut microbiota in atherosclerosis. Nat. Rev. Cardiol. 14, 79–87. https://doi.org/10.1038/nrcardio.2016.183.

Joosten, M.M., Pai, J.K., Bertoia, M.L., Rimm, E.B., Spiegelman, D., Mittleman, M.A., Mukamal, K.J., 2012. Associations between conventional cardiovascular risk factors and risk of peripheral artery disease in men. JAMA 308, 1660–1667. https://doi.org/10.1001/jama.2012.13415.

Kaplanski, G., 2018. Interleukin-18: biological properties and role in disease pathogenesis. Immunol. Rev. 281, 138–153. https://doi.org/10.1111/imr.12616.

Karlsson, F.H., Fåk, F., Nookaew, I., Tremaroli, V., Fagerberg, B., Petranovic, D., Bäckhed, F., Nielsen, J., 2012. Symptomatic atherosclerosis is associated with an altered gut metagenome. Nat. Commun. 3, 1245. https://doi.org/10.1038/ncomms2266.

Karunakaran, D., Thrush, A.B., Nguyen, M.-A., Richards, L., Geoffrion, M., Singaravelu, R., Ramphos, E., Shangari, P., Ouimet, M., Pezacki, J.P., Moore, K.J., Perisic, L., Maegdefessel, L., Hedin, U., Harper, M.-E., Rayner, K.J., 2015. Macrophage mitochondrial energy status regulates cholesterol efflux and is enhanced by Anti-miR33 in atherosclerosis. Circ. Res. 117, 266–278. https://doi.org/10.1161/CIRCRESAHA.117.305624.

Karunakaran, D., Geoffrion, M., Wei, L., Gan, W., Richards, L., Shangari, P., DeKemp, E.M., Beanlands, R.A., Perisic, L., Maegdefessel, L., Hedin, U., Sad, S., Guo, L., Kolodgie, F.D., Virmani, R., Ruddy, T., Rayner, K.J., 2016. Targeting macrophage necroptosis for therapeutic and diagnostic interventions in atherosclerosis. Sci. Adv. 2. https://doi.org/10.1126/sciadv.1600224.

Katsuumi, G., Shimizu, I., Yoshida, Y., Minamino, T., 2018. Vascular senescence in cardiovascular and metabolic diseases. Front. Cardiovasc. Med. 5. https://doi.org/10.3389/fcvm.2018.00018.

Kawanishi, K., Dhar, C., Do, R., Varki, N., Gordts, P.L.S.M., Varki, A., 2019. Human species-specific loss of CMP-N-acetylneuraminic acid hydroxylase enhances atherosclerosis via intrinsic and extrinsic mechanisms. PNAS 2019, 02902. https://doi.org/10.1073/pnas.1902902116.

Kennedy, B.K., Berger, S.L., Brunet, A., Campisi, J., Cuervo, A.M., Epel, E.S., Franceschi, C., Lithgow, G.J., Morimoto, R.I., Pessin, J.E., Rando, T.A., Richardson, A., Schadt, E.E., Wyss-Coray, T., Sierra, F., 2014. Geroscience: linking aging to chronic disease. Cell 159, 709–713. https://doi.org/10.1016/j.cell.2014.10.039.

Kooman, J.P., Dekker, M.J., Usvyat, L.A., Kotanko, P., van der Sande, F.M., Schalkwijk, C.G., Shiels, P.G., Stenvinkel, P., 2017. Inflammation and premature aging in advanced chronic kidney disease. Am. J. Physiol. Renal Physiol. 313, F938–F950. https://doi.org/10.1152/ajprenal.00256.2017.

Kotsis, V.T., Stabouli, S.V., Papamichael, C.M., Zakopoulos, N.A., 2006. Impact of Obesity in Intima media Thickness of Carotid Arteries. Obesity 14. Silver Spring, pp. 1708–1715. https://doi.org/10.1038/oby.2006.196.

Kraus, W.E., Bhapkar, M., Huffman, K.M., et al., 2019. 2 years of calorie restriction and cardiometabolic risk (CALERIE): exploratory outcomes of a multicentre, phase 2, randomised controlled trial. Lancet Diabetes Endocrinol. 7 (9), 673–683. https://doi.org/10.1016/S2213-8587(19)30151-2.

Laffont, B., Rayner, K.J., 2017. MicroRNAs in the pathobiology of atherosclerosis. Can. J. Cardiol. 33, 313–324. https://doi.org/10.1016/j.cjca.2017.01.001.

Lakkur, S., Judd, S.E., 2015. Diet and stroke. Stroke 46, 2007–2011. https://doi.org/10.1161/STROKEAHA.114.006306.

Lau, K.-K., Chan, Y.-H., Yiu, K.-H., Li, S.-W., Tam, S., Lau, C.-P., Kwong, Y.-L., Tse, H.-F., 2007. Burden of carotid atherosclerosis in patients with stroke: relationships with circulating endothelial progenitor cells and hypertension. J. Hum. Hypertens. 21, 445–451. https://doi.org/10.1038/sj.jhh.1002178.

Li, X., Ploner, A., Wang, Y., Magnusson, P.K., Reynolds, C., Finkel, D., Pedersen, N.L., Jylhävä, J., Hägg, S., 2020. Longitudinal trajectories, correlations and mortality associations of nine biological ages across 20-years follow-up. eLife 9, e51507. https://doi.org/10.7554/eLife.51507.

Libby, P., Buring, J.E., Badimon, L., Hansson, G.K., Deanfield, J., Bittencourt, M.S., Tokgözoğlu, L., Lewis, E.F., 2019. Atherosclerosis. Nature Reviews Disease Primers 5. pp. 56. https://doi.org/10.1038/s41572-019-0106-z.

Liberale, L., Montecucco, F., Tardif, J.-C., Libby, P., Camici, G.G., 2020. Inflamm-ageing: the role of inflammation in age-dependent cardiovascular disease [published online ahead of print, 2020 Feb 1]. Eur. Heart J., ehz961. https://doi.org/10.1093/eurheartj/ehz961.

Lin, Y., Liu, X., Cheng, Y., Yang, J., Huo, Y., Zhang, C., 2009. Involvement of MicroRNAs in hydrogen peroxide-mediated gene regulation and cellular injury response in vascular smooth muscle cells. J. Biol. Chem. 284, 7903–7913. https://doi.org/10.1074/

jbc.M806920200.
Linz, W., Heitsch, H., Schölkens, B.A., Wiemer, G., 2000. Long-term angiotensin II type 1 receptor blockade with fonsartan doubles lifespan of hypertensive rats. Hypertension 35, 908–913. https://doi.org/10.1161/01.HYP.35.4.908.
Liu, H., Cao, Y., Tong, T., Shi, J., Zhang, Y., Yang, Y., Liu, C., 2015. Autophagy in atherosclerosis: a phenomenon found in human carotid atherosclerotic plaques. Chin. Med. J. 128, 69–74. https://doi.org/10.4103/0366-6999.147815.
Lovren, Fina, Yi, Pan, Adrian, Quan, Singh Krishna, K., Shukla Praphulla, C., Nandini, Gupta, Steer Brent, M., Ingram Alistair, J., Milan, Gupta, Mohammed, Al-Omran, Hwee, Teoh, Marsden Philip, A., Subodh, Verma, 2012. MicroRNA-145 targeted therapy reduces atherosclerosis. Circulation 126, S81–S90. https://doi.org/10.1161/CIRCULATIONAHA.111.084186.
Luque, A., Farwati, A., Krupinski, J., Aran, J.M., 2018. Association between low levels of serum miR-638 and atherosclerotic plaque vulnerability in patients with high-grade carotid stenosis. J. Neurosurg. 1–8. https://doi.org/10.3171/2018.2.JNS171899.
Lusis, A.J., 2000. Atherosclerosis. Nature 407, 233. https://doi.org/10.1038/35025203.
Magenta, A., Sileno, S., D'Agostino, M., Persiani, F., Beji, S., Paolini, A., Camilli, D., Platone, A., Capogrossi, M.C., Furgiuele, S., 2018. Atherosclerotic plaque instability in carotid arteries: miR-200c as a promising biomarker. Clin. Sci. 132, 2423–2436. https://doi.org/10.1042/CS20180684.
Maitrias, P., Metzinger-Le Meuth, V., Massy, Z.A., M'Baya-Moutoula, E., Reix, T., Caus, T., Metzinger, L., 2015. MicroRNA deregulation in symptomatic carotid plaque. J. Vasc. Surg. 62, 1245–1250. https://doi.org/10.1016/j.jvs.2015.06.136. e1.
Maitrias, P., Metzinger-Le Meuth, V., Nader, J., Reix, T., Caus, T., Metzinger, L., 2017. The involvement of miRNA in carotid-related stroke. Arterioscler. Thromb. Vasc. Biol. 37, 1608–1617. https://doi.org/10.1161/ATVBAHA.117.309233.
Marfella, R., D'Amico, M., Di Filippo, C., Baldi, A., Siniscalchi, M., Sasso, F.C., Portoghese, M., Carbonara, O., Crescenzi, B., Sangiuolo, P., Nicoletti, G.F., Rossiello, R., Ferraraccio, F., Cacciapuoti, F., Verza, M., Coppola, L., Rossi, F., Paolisso, G., 2006. Increased activity of the ubiquitin-proteasome system in patients with symptomatic carotid disease is associated with enhanced inflammation and may destabilize the atherosclerotic plaque: effects of rosiglitazone treatment. J. Am. Coll. Cardiol. 47, 2444–2455. https://doi.org/10.1016/j.jacc.2006.01.073.
Markus, B., Grote, K., Worsch, M., Parviz, B., Boening, A., Schieffer, B., Parahuleva, M.S., 2016. Differential expression of MicroRNAs in endarterectomy specimens taken from patients with asymptomatic and symptomatic carotid plaques. PLoS One 11, e0161632. https://doi.org/10.1371/journal.pone.0161632.
Marnane, M., Prendeville, S., McDonnell, C., Noone, I., Barry, M., Crowe, M., Mulligan, N., Kelly, P.J., 2014. Plaque inflammation and unstable morphology are associated with early stroke recurrence in symptomatic carotid stenosis. Stroke 45, 801–806. https://doi.org/10.1161/STROKEAHA.113.003657.
Martinet, Wim, Knaapen Michiel, W.M., De Meyer Guido, R.Y., Herman Arnold, G., Kockx Mark, M., 2002. Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. Circulation 106, 927–932. https://doi.org/10.1161/01.CIR.0000026393.47805.21.
Martinez, E., Martorell, J., Riambau, V., 2020. Review of serum biomarkers in carotid atherosclerosis. J. Vasc. Surg. 71, 329–341. https://doi.org/10.1016/j.jvs.2019.04.488.
Mathiesen, E.B., Joakimsen, O., Bønaa, K.H., 2001. Prevalence of and risk factors associated with carotid artery stenosis: the Tromsø Study. Cerebrovasc. Dis. 12, 44–51. https://doi.org/10.1159/000047680.
Mattagajasingh, I., Kim, C.-S., Naqvi, A., Yamamori, T., Hoffman, T.A., Jung, S.-B., DeRicco, J., Kasuno, K., Irani, K., 2007. SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. Proc Natl Acad Sci U S A 104, 14855–14860. https://doi.org/10.1073/pnas.0704329104.
Matthews, Charles, et al., 2006. Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis. Circ. Res. 99, 156–164. https://doi.org/10.1161/01.RES.0000233315.38086.bc.
Matturri, L., Cazzullo, A., Turconi, P., Lavezzi, A.M., Vandone, P.L., Gabrielli, L., Fernández Alonso, G., Grana, D., Milei, J., 2001. Chromosomal alterations in atherosclerotic plaques. Atherosclerosis 154, 755–761.
McLaren, J.E., Michael, D.R., Ashlin, T.G., Ramji, D.P., 2011. Cytokines, macrophage lipid metabolism and foam cells: implications for cardiovascular disease therapy. Prog. Lipid Res. 50, 331–347. https://doi.org/10.1016/j.plipres.2011.04.002.
Metzinger-Le Meuth, V., Burtey, S., Maitrias, P., Massy, Z.A., Metzinger, L., 2017. microRNAs in the pathophysiology of CKD-MBD: biomarkers and innovative drugs. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1863, 337–345. https://doi.org/10.1016/j.bbadis.2016.10.027.
Moore, K.J., Sheedy, F.J., Fisher, E.A., 2013. Macrophages in atherosclerosis: a dynamic balance. Nat. Rev. Immunol. 13, 709–721. https://doi.org/10.1038/nri3520.
Morsiani, C., Bacalini, M.G., Santoro, A., Garagnani, P., Collura, S., D'Errico, A., de Eguileor, M., Grazi, G.L., Cescon, M., Franceschi, C., Capri, M., 2019. The peculiar aging of human liver: a geroscience perspective within transplant context. Ageing Res. Rev. 51, 24–34. https://doi.org/10.1016/j.arr.2019.02.002.
Moskalev, A., 2020. The challenges of estimating biological age. eLife 9, e54969. https://doi.org/10.7554/eLife.54969.
Moss, J.W.E., Ramji, D.P., 2016. Cytokines: roles in atherosclerosis disease progression and potential therapeutic targets. Future Med. Chem. 8, 1317–1330. https://doi.org/10.4155/fmc-2016-0072.
Najafi-Shoushtari, S.H., Kristo, F., Li, Y., Shioda, T., Cohen, D.E., Gerszten, R.E., Näär, A.M., 2010. MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis. Science 328, 1566–1569. https://doi.org/10.1126/science.1189123.
Nash, S.D., Cruickshanks, K.J., Klein, R., Klein, B.E.K., Nieto, F.J., Ryff, C.D., Krantz, E.M., Shubert, C.R., Nondahl, D.M., Acher, C.W., 2011. Socioeconomic status and subclinical atherosclerosis in older adults. Prev. Med. 52, 208–212. https://doi.org/10.1016/j.ypmed.2010.12.009.
Naylor, A.R., Ricco, et al., 2018. Editor's choice – management of atherosclerotic carotid and vertebral artery disease: 2017 clinical practice guidelines of the european society for vascular surgery (ESVS). Eur. J. Vasc. Endovasc. Surg. 55, 3–81. https://doi.org/10.1016/j.ejvs.2017.06.021.
North, B.J., Sinclair, D.A., 2012. The intersection between aging and cardiovascular disease. Circ. Res. 110, 1097–1108. https://doi.org/10.1161/CIRCRESAHA.111.246876.
O'Leary, D.H., Polak, J.F., Kronmal, R.A., Kittner, S.J., Bond, M.G., Wolfson, S.K., Bommer, W., Price, T.R., Gardin, J.M., Savage, P.J., 1992. Distribution and correlates of sonographically detected carotid artery disease in the Cardiovascular Health Study. The CHS Collaborative Research Group. Stroke 23, 1752–1760.
Olivieri, F., Spazzafumo, L., Santini, G., Lazzarini, R., Albertini, M.C., Rippo, M.R., Galeazzi, R., Abbatecola, A.M., Marcheselli, F., Monti, D., Ostan, R., Cevenini, E., Antonicelli, R., Franceschi, C., Procopio, A.D., 2012. Age-related differences in the expression of circulating microRNAs: miR-21 as a new circulating marker of inflammaging. Mech. Ageing Dev. 133, 675–685. https://doi.org/10.1016/j.mad.2012.09.004.
Olivieri, F., Rippo, M.R., Procopio, A.D., Fazioli, F., 2013. Circulating inflamma-miRs in aging and age-related diseases. Front. Genet. 4. https://doi.org/10.3389/fgene.2013.00121.
Olivieri, F., Capri, M., Bonafè, M., Morsiani, C., Jung, H.J., Spazzafumo, L., Viña, J., Suh, Y., 2017. Circulating miRNAs and miRNA shuttles as biomarkers: perspective trajectories of healthy and unhealthy aging. Mech. Ageing Dev. 165, 162–170. https://doi.org/10.1016/j.mad.2016.12.004.
Ouimet, M., Ediriweera, H.N., Gundra, U.M., Sheedy, F.J., Ramkhelawon, B., Hutchison, S.B., Rinehold, K., van Solingen, C., Fullerton, M.D., Cecchini, K., Rayner, K.J., Steinberg, G.R., Zamore, P.D., Fisher, E.A., Loke, P., Moore, K.J., 2015. MicroRNA-33-dependent regulation of macrophage metabolism directs immune cell polarization in atherosclerosis. J. Clin. Invest. 125, 4334–4348. https://doi.org/10.1172/JCI81676.
Palanca, A., Castelblanco, E., Perpiñán, H., Betriu, À., Soldevila, B., Valdivielso, J.M., Bermúdez, M., Duran, X., Fernández, E., Puig-Domingo, M., Groop, P.-H., Alonso, N., Mauricio, D., 2018. Prevalence and progression of subclinical atherosclerosis in patients with chronic kidney disease and diabetes. Atherosclerosis 276, 50–57. https://doi.org/10.1016/j.atherosclerosis.2018.07.018.
Pelisek, J., Rudelius, M., Zepper, P., Poppert, H., Reeps, C., Schuster, T., Eckstein, H.-H., 2009. Multiple biological predictors for vulnerable carotid lesions. Cerebrovasc. Dis. 28, 601–610. https://doi.org/10.1159/000247605.
Pelisek, J., Eckstein, H.-H., Zernecke, A., 2012. Pathophysiological mechanisms of carotid plaque vulnerability: impact on ischemic stroke. Arch. Immunol. Ther. Exp. (Warsz.) 60, 431–442. https://doi.org/10.1007/s00005-012-0192-z.
Peng, H., Zhong, M., Zhao, W., Wang, C., Zhang, J., Liu, X., Li, Y., Paudel, S.D., Wang, Q., Lou, T., 2013. Urinary miR-29 correlates with Albuminuria and carotid intima-media thickness in type 2 diabetes patients. PLoS One 8, e82607. https://doi.org/10.1371/journal.pone.0082607.
Pini, R., Faggioli, G., Fittipaldi, S., Vasuri, F., Longhi, M., Gallitto, E., Pasquinelli, G., Gargiulo, M., Stella, A., 2017. Relationship between calcification and vulnerability of the carotid plaques. Ann. Vasc. Surg. 44, 336–342. https://doi.org/10.1016/j.avsg.2017.04.017.
Pyrkov, T.V., Fedichev, P.O., 2019. Biological age is a universal marker of aging, stress, and frailty. bioRxiv, 578245. https://doi.org/10.1101/578245.
Raitoharju, E., Lyytikäinen, L.-P., Levula, M., Oksala, N., Mennander, A., Tarkka, M., Klopp, N., Illig, T., Kähönen, M., Karhunen, P.J., Laaksonen, R., Lehtimäki, T., 2011. miR-21, miR-210, miR-34a, and miR-146a/b are up-regulated in human atherosclerotic plaques in the Tampere Vascular Study. Atherosclerosis 219, 211–217. https://doi.org/10.1016/j.atherosclerosis.2011.07.020.
Ramji, D.P., Davies, T.S., 2015. Cytokines in atherosclerosis: key players in all stages of disease and promising therapeutic targets. Cytokine Growth Factor Rev. 26, 673–685. https://doi.org/10.1016/j.cytogfr.2015.04.003.
Rayner, K.J., Suárez, Y., Dávalos, A., Parathath, S., Fitzgerald, M.L., Tamehiro, N., Fisher, E.A., Moore, K.J., Fernández-Hernando, C., 2010. MiR-33 contributes to the regulation of cholesterol homeostasis. Science 328, 1570–1573. https://doi.org/10.1126/science.1189862.
Rea, I.M., Gibson, D.S., McGilligan, V., McNerlan, S.E., Alexander, H.D., Ross, O.A., 2018. Age and age-related diseases: role of inflammation triggers and cytokines. Front. Immunol. 9. https://doi.org/10.3389/fimmu.2018.00586.
Redgrave, J.N.E., Lovett, J.K., Rothwell, P.M., 2010. Histological features of symptomatic carotid plaques in relation to age and smoking: the oxford plaque study. Stroke 41, 2288–2294. https://doi.org/10.1161/STROKEAHA.110.587006.
Roos, C.M., Zhang, B., Palmer, A.K., Ogrodnik, M.B., Pirtskhalava, T., Thalji, N.M., Hagler, M., Jurk, D., Smith, L.A., Casaclang-Verzosa, G., Zhu, Y., Schafer, M.J., Tchkonia, T., Kirkland, J.L., Miller, J.D., 2016. Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. Aging Cell 15, 973–977. https://doi.org/10.1111/acel.12458.
Rose, G., Romeo, G., Dato, S., Crocco, P., Bruni, A.C., Hervonen, A., Majamaa, K., Sevini, F., Franceschi, C., Passarino, G., Consortium, G.P., 2010. Somatic point mutations in mtDNA control region are influenced by genetic background and associated with healthy aging: a GEHA study. PLoS One 5, e13395. https://doi.org/10.1371/journal.pone.0013395.
Ross, R., 1999. Atherosclerosis — an inflammatory disease. N. Engl. J. Med. 340, 115–126. https://doi.org/10.1056/NEJM199901143400207.
Roth, G.A., et al., 2018. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392, 1736–1788. https://doi.org/10.1016/S0140-6736(18)32203-7.
Rubio-Ruiz, M.E., Pérez-Torres, I., Soto, M.E., Pastelín, G., Guarner-Lans, V., 2014. Aging

in blood vessels. Medicinal agents FOR systemic arterial hypertension in the elderly. Ageing Res. Rev. 18, 132–147. https://doi.org/10.1016/j.arr.2014.10.001.
Santovito, D., Mandolini, C., Marcantonio, P., Nardis, V.D., Bucci, M., Paganelli, C., Magnacca, F., Ucchino, S., Mastroiacovo, D., Desideri, G., Mezzetti, A., Cipollone, F., 2013. Overexpression of microRNA-145 in atherosclerotic plaques from hypertensive patients. Expert Opin. Ther. Targets 17, 217–223. https://doi.org/10.1517/14728222.2013.745512.
Schillinger, M., Exner, M., Mlekusch, W., Sabeti, S., Amighi, J., Nikowitsch, R., Timmel, E., Kickinger, B., Minar, C., Pones, M., Lalouschek, W., Rumpold, H., Maurer, G., Wagner, O., Minar, E., 2005. Inflammation and carotid artery–Risk for atherosclerosis study (ICARAS). Circulation 111, 2203–2209. https://doi.org/10.1161/01.CIR.0000163569.97918.C0.
Scholtes, V.P.W., Peeters, W., van Lammeren, G.W., Howard, D.P.J., de Vries, J.-P.P.M., de Borst, G.J., Redgrave, J.N., Kemperman, H., Schalkwijk, C.G., den Ruijter, H.M., de Kleijn, D.P.V., Moll, F.L., Rothwell, P.M., Pasterkamp, G., 2014. Type 2 diabetes is not associated with an altered plaque phenotype among patients undergoing carotid revascularization. A histological analysis of 1455 carotid plaques. Atherosclerosis 235, 418–423. https://doi.org/10.1016/j.atherosclerosis.2014.05.941.
Scuteri, A., Tesauro, M., Rizza, S., Iantorno, M., Federici, M., Lauro, D., Campia, U., Turriziani, M., Fusco, A., Cocciolillo, G., Lauro, R., 2008. Endothelial function and arterial stiffness in normotensive normoglycemic first-degree relatives of diabetic patients are independent of the metabolic syndrome. Nutr. Metab. Cardiovasc. Dis. 18, 349–356. https://doi.org/10.1016/j.numecd.2007.03.008.
Shah, A., Gray, K., Figg, N., Finigan, A., Starks, L., Bennett, M., 2018. Defective base excision repair of oxidative DNA damage in vascular smooth muscle cells promotes atherosclerosis. Circulation 138, 1446–1462. https://doi.org/10.1161/CIRCULATIONAHA.117.033249.
Shi, X., Xie, W.-L., Kong, W.-W., Chen, D., Qu, P., 2015. Expression of the NLRP3 inflammasome in carotid atherosclerosis. J. Stroke Cerebrovasc. Dis. 24, 2455–2466. https://doi.org/10.1016/j.jstrokecerebrovasdis.2015.03.024.
Siemelink Marten, A., et al., 2018. Smoking is associated to DNA methylation in atherosclerotic carotid lesions. Circulation: Genomic and Precision Medicine 11, e002030. https://doi.org/10.1161/CIRCGEN.117.002030.
Sigala, F., Oikonomou, E., Antonopoulos, A.S., Galyfos, G., Tousoulis, D., 2018. Coronary versus carotid artery plaques. Similarities and differences regarding biomarkers morphology and prognosis. Curr. Opin. Pharmacol. 39, 9–18. https://doi.org/10.1016/j.coph.2017.11.010.
Skagen, K., Trøseid, M., Ueland, T., Holm, S., Abbas, A., Gregersen, I., Kummen, M., Bjerkeli, V., Reier-Nilsen, F., Russell, D., Svardal, A., Karlsen, T.H., Aukrust, P., Berge, R.K., Hov, J.E.R., Halvorsen, B., Skjelland, M., 2016. The Carnitine-butyrobetaine-trimethylamine-N-oxide pathway and its association with cardiovascular mortality in patients with carotid atherosclerosis. Atherosclerosis 247, 64–69. https://doi.org/10.1016/j.atherosclerosis.2016.01.033.
Slovut, D.P., Olin, J.W., 2004. Fibromuscular dysplasia. N. Engl. J. Med. 350, 1862–1871. https://doi.org/10.1056/NEJMra032393.
Stankovic, M., Ljujic, B., Babic, S., Maravic-Stojkovic, V., Mitrovic, S., Arsenijevic, N., Radak, D., Pejnovic, N., Lukic, M.L., 2019. IL-33/IL-33R in various types of carotid artery atherosclerotic lesions. Cytokine 120, 242–250. https://doi.org/10.1016/j.cyto.2019.05.010.
Stein, S., Matter, C.M., 2011. Protective roles of SIRT1 in atherosclerosis. Cell Cycle 10, 640–647. https://doi.org/10.4161/cc.10.4.14863.
Swaminathan, B., Goikuria, H., Vega, R., Rodríguez-Antigüedad, A., Medina, A.L., Freijo, M., del, M., Vandenbroeck, K., Alloza, I., 2014. Autophagic marker MAP1LC3B expression levels are associated with carotid atherosclerosis symptomatology. PLoS One 9, e115176. https://doi.org/10.1371/journal.pone.0115176.
Tabas, I., 2010. The role of endoplasmic reticulum stress in the progression of atherosclerosis. Circ. Res. 107, 839–850. https://doi.org/10.1161/CIRCRESAHA.110.224766.
Tabas, I., Lichtman, A.H., 2017. Monocyte-macrophages and t cells in atherosclerosis. Immunity 47, 621–634. https://doi.org/10.1016/j.immuni.2017.09.008.
Thurston Rebecca, C., El Khoudary Samar, R., Derby Carol, A., Emma, Barinas-Mitchell, Lewis Tené, T., McClure Candace, K., Matthews Karen, A., 2014. Low socioeconomic status over 12 years and subclinical cardiovascular disease. Stroke 45, 954–960. https://doi.org/10.1161/STROKEAHA.113.004162.
Tomas, L., Edsfeldt, A., Mollet, I.G., Perisic Matic, L., Prehn, C., Adamski, J., Paulsson-Berne, G., Hedin, U., Nilsson, J., Bengtsson, E., Gonçalves, I., Björkbacka, H., 2018. Altered metabolism distinguishes high-risk from stable carotid atherosclerotic plaques. Eur. Heart J. 39, 2301–2310. https://doi.org/10.1093/eurheartj/ehy124.
Tsai, P.-C., Liao, Y.-C., Wang, Y.-S., Lin, H.-F., Lin, R.-T., Juo, S.-H.H., 2013. Serum microRNA-21 and microRNA-221 as potential biomarkers for cerebrovascular disease. JVR 50, 346–354. https://doi.org/10.1159/000351767.
Tzoulaki, I., et al., 2019. Serum metabolic signatures of coronary and carotid atherosclerosis and subsequent cardiovascular disease. Eur. Heart J. 40, 2883–2896. https://doi.org/10.1093/eurheartj/ehz235.
Uslu, B., Cakmak, Y.O., Sehirli, Ü., Keskinoz, E.N., Cosgun, E., Arbak, S., Yalin, A., 2016. Early onset of atherosclerosis of the carotid bifurcation in newborn cadavers. J. Clin. Diagn. Res. 10, AC01–AC05. https://doi.org/10.7860/JCDR/2016/19827.7706.
Valdivielso, J.M., Rodríguez-Puyol, D., Pascual, J., Barrios, C., Bermúdez-López, M., Sánchez-Niño, M.D., Pérez-Fernández, M., Ortiz, A., 2019. Atherosclerosis in Chronic Kidney Disease. Arterioscler. Thromb. Vasc. Biol. 39, 1938–1966. https://doi.org/10.1161/ATVBAHA.119.312705.
Vasuri, F., Fittipaldi, S., Pasquinelli, G., 2014. Arterial calcification: finger-pointing at resident and circulating stem cells. World J. Stem Cells 6, 540–551. https://doi.org/10.4252/wjsc.v6.i5.540.
Vasuri, F., Ciavarella, C., Fittipaldi, S., Pini, R., Vacirca, A., Gargiulo, M., Faggioli, G., Pasquinelli, G., 2019. Different histological types of active intraplaque calcification underlie alternative miRNA-mRNA axes in carotid atherosclerotic disease. Virchows Arch. https://doi.org/10.1007/s00428-019-02659-w.
Vendrov, A.E., Vendrov, K.C., Smith, A., Yuan, J., Sumida, A., Robidoux, J., Runge, M.S., Madamanchi, N.R., 2015. NOX4 NADPH oxidase-dependent mitochondrial oxidative stress in aging-associated cardiovascular disease. Antioxid. Redox Signal. 23, 1389–1409. https://doi.org/10.1089/ars.2014.6221.
Versari, D., Herrmann, J., Gössl, M., Mannheim, D., Sattler, K., Meyer, F.B., Lerman, L.O., Lerman, A., 2006. Dysregulation of the ubiquitin-proteasome system in human carotid atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 26, 2132–2139. https://doi.org/10.1161/01.ATV.0000232501.08576.73.
Wang, Z., Klipfell, E., Bennett, B.J., Koeth, R., Levison, B.S., DuGar, B., Feldstein, A.E., Britt, E.B., Fu, X., Chung, Y.-M., Wu, Y., Schauer, P., Smith, J.D., Allayee, H., Tang, W.H.W., DiDonato, J.A., Lusis, A.J., Hazen, S.L., 2011. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 472, 57–63. https://doi.org/10.1038/nature09922.
Warnatsch, A., Ioannou, M., Wang, Q., Papayannopoulos, V., 2015. Neutrophil extracellular traps license macrophages and Th17 cells for cytokine production in atherosclerosis. Science 349, 316–320. https://doi.org/10.1126/science.aaa8064.
Wild, C.P., 2012. The exposome: from concept to utility. Int. J. Epidemiol. 41, 24–32. https://doi.org/10.1093/ije/dyr236.
World Health Organization, 2017. Cardiovascular Diseases (CVDs). https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
Xu, W., Gao, L., Zheng, J., Li, T., Shao, A., Reis, C., Chen, S., Zhang, J., 2018. The roles of MicroRNAs in stroke: possible therapeutic targets. Cell Transplant. 27, 1778–1788. https://doi.org/10.1177/0963689718773361.
Yahagi, K., Kolodgie, F.D., Lutter, C., Mori, H., Romero, M.E., Finn, A.V., Virmani, R., 2017. Pathology of human coronary and carotid artery atherosclerosis and vascular calcification in diabetes mellitus. Arterioscler. Thromb. Vasc. Biol. 37, 191–204. https://doi.org/10.1161/ATVBAHA.116.306256.
Yao, Lu, Tanuja, Thavarajah, Wenduo, Gu, Jingjing, Cai, Qingbo, Xu, 2018. Impact of miRNA in atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 38, e159–e170. https://doi.org/10.1161/ATVBAHA.118.310227.
Yizhak, K., Aguet, F., Kim, J., Hess, J.M., Kübler, K., Grimsby, J., Frazer, R., Zhang, H., Haradhvala, N.J., Rosebrock, D., Livitz, D., Li, X., Arich-Landkof, E., Shoresh, N., Stewart, C., Segrè, A.V., Branton, P.A., Polak, P., Ardlie, K.G., Getz, G., 2019. RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues. Science 364, eaaw0726. https://doi.org/10.1126/science.aaw0726.
Yuan, X.-M., Osman, E., Miah, S., Zadeh, S.N.M., Xu, L., Forssell, C., Li, W., 2010. p53 expression in human carotid atheroma is significantly related to plaque instability and clinical manifestations. Atherosclerosis 210, 392–399. https://doi.org/10.1016/j.atherosclerosis.2009.11.048.
Zernecke, A., Bidzhekov, K., Noels, H., Shagdarsuren, E., Gan, L., Denecke, B., Hristov, M., Köppel, T., Jahantigh, M.N., Lutgens, E., Wang, S., Olson, E.N., Schober, A., Weber, C., 2009. Delivery of MicroRNA-126 by apoptotic bodies induces CXCL12-Dependent vascular protection. Sci. Signal. 2, ra81. https://doi.org/10.1126/scisignal.2000610.
Zhang, R., Qin, Y., Zhu, G., Li, Y., Xue, J., 2016. Low serum miR-320b expression as a novel indicator of carotid atherosclerosis. J. Clin. Neurosci. 33, 252–258. https://doi.org/10.1016/j.jocn.2016.03.034.
Zhaolin, Z., Guohua, L., Shiyuan, W., Zuo, W., 2019. Role of pyroptosis in cardiovascular disease. Cell Prolif. 52, e12563. https://doi.org/10.1111/cpr.12563.
Zhu, Y., et al., 2015. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell 14, 644–658. https://doi.org/10.1111/acel.12344.
Zureik, M., Czernichow, S., Courbon, D., Blacher, J., Ducimetière, P., Hercberg, S., Safar, M.E., Galan, P., 2006. Parental longevity, carotid atherosclerosis, and aortic arterial stiffness in adult offspring. Stroke 37, 2702–2707. https://doi.org/10.1161/01.STR.0000244759.62619.83.