

RESEARCH HIGHLIGHT

MicroRNA-34a: a new player in arterial inflammaging

Ileana Badi, Angela Raucci

Experimental Cardio-Oncology and Cardiovascular Aging Unit, Centro Cardiologico Monzino-IRCCS, Milan, Italy

Correspondence: Angela Raucci

E-mail: araucci@ccfm.it

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Arterial inflammaging highly contributes to cardiovascular morbidity and mortality. As vascular cells age they become senescent and sustain a chronic low grade sterile inflammation by acquiring a senescence-associated secretory phenotype (SASP). The molecular mechanisms leading to the phenotypic changes affecting endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) are also relevant for the pathogenesis of vascular diseases, such as atherosclerosis and hypertension. Therefore, unravelling the etiology of vascular inflammaging becomes of crucial importance. MicroRNAs (miRNAs) are small non-coding negative post-transcriptional regulator that are emerging as promising drug targets. MicroRNA-34a (miR-34a) had been implicated in tissues aging and endothelial and endothelial progenitor cells senescence. Our recent work showed that this miRNA is upregulated in aged mouse aortas as well as in senescent VSMCs. Conversely, its target SIRT1 is downregulated in the same specimens. We also found that miR-34a can inhibit VSMCs proliferation and induce VSMCs senescence, the latter by the direct regulation of SIRT1. Notably, for the first time, we demonstrated that miR-34a is also able to modulate the SASP by inducing the transcriptional expression of a subset of pro-inflammatory factors in a SIRT1-independent manner. These data support a model in which the age-dependent upregulation of miR-34a, by affecting senescence and inflammation of vascular cells, could play a causal role to arterial dysfunctions. Hence, further studies are necessary to unravel miR-34a-dependent mechanisms leading to arterial inflammaging in order to develop an effective strategy for age-related cardiovascular complications.

Keywords: miR-34a; Inflammaging; SIRT1; SASP; Vascular Aging

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The term “inflammaging” was coined 15 years ago to describe the chronic, low-grade systemic inflammation that occurs during physiological aging in the absence of an overt infection ^[1]. Although inflammaging is a hallmark of all age-associated diseases and highly contributes to morbidity and mortality in the elderly population ^[1], its etiology remains largely unknown ^[2]. Thus, unravelling the molecular mechanisms which trigger and control inflammaging is extremely important, as it could help in the development of effective treatments for almost all the age-related diseases.

Likewise arterial aging, that is an important risk factor for cardiovascular morbidity and mortality ^[3], begins with persistent low grade “sterile” inflammation ^[4] and cellular

senescence is emerging as an important contributor to its maintenance via the acquisition of a senescence-associated secretory phenotype (SASP). Indeed, vascular cells release soluble molecules associated to inflammation, growth and extracellular matrix remodeling, such as cytokines chemokines, adhesion molecules, growth factors and proteases, that acting in an autocrine and paracrine manner, influence neighboring cells behavior altering their microenvironment ^[4,5]. Both the main cell types constituting the arterial wall undergo these age-related phenotypic changes and the mechanisms that lead to these alterations, namely endothelial dysfunction and vascular smooth muscle cells (VSMCs) phenotypic shifts, are fundamental for the

pathogenesis of vascular diseases such as atherosclerosis and hypertension^[4]. Therefore, elucidating the cellular and molecular processes underlying vascular inflammaging and related dysfunctions is of high scientific interest.

MicroRNAs (miRNAs) are small non-coding RNA molecules that usually negatively regulate the post-transcriptional expression levels of multiple target genes and they are emerging as a promising class of drug targets^[6]. MicroRNA-34a (miRNA-34a) was firstly described as a tumor suppressor miRNA, direct target of p53 that, when up-regulated, can induce apoptosis, cell cycle arrest and senescence in several cancers^[7, 8]. Recently miRNA-34a expression have been shown to increase with age in different tissues and organs of humans and mice^[9-11]. Notably, miR-34a levels increase with aging in human peripheral blood and murine plasma and peripheral blood mononuclear cells (PBMCs); furthermore, Schipper and colleagues found augmented miR-34a levels in PBMCs of patients with the inflammaging-associated Alzheimer's disease compared to normal elderly controls^[9, 12-14]. Concerning vascular cells, Ito and collaborators demonstrated that miR-34a can induce endothelial cells (ECs) senescence at least in part through the modulation of the longevity-associated SIRT1 gene^[15] and Zhao and co-workers observed increased senescence and decreased SIRT1 protein levels upon miR-34a overexpression in endothelial progenitor cells^[16]. However, the miR-34a role in whole aorta and specifically VSMCs aging and associated inflammation has been unexplored so far.

We, therefore, undertook a study aimed to understand whether miR-34a could affect VSMCs senescence and their SASP^[17]. Firstly, we demonstrated that miR-34a expression levels were increased in aged aortas of old mice compared to young animals while the contractile smooth muscle cells marker (SM22a) appeared downregulated. Interestingly, among all the assessed miR-34a targets (SIRT1, Bcl2 and Axl) only SIRT1 protein levels were significantly downregulated during aging. We also observed that SIRT1 was expressed in both ECs and VSMCs in young aortas, while it was barely detectable in both cell types in the aged vessels. In accordance to the *in vivo* data, miR-34a expression increased whereas SIRT1 protein levels decreased during replicative senescence of cultured VSMCs. Moreover, overexpression of miR-34a in proliferative VSMCs caused at first, G0/G1 cell-cycle arrest along with p21 upregulation, followed by SIRT1 downregulation and senescence. While miR-34a-induced inhibition of VSMCs proliferation was SIRT1-independent, induction of cell senescence resulted, at least in part, directly regulated by this sirtuin. These results suggest that miR-34a could be an important player in vascular aging and its regulation of senescence as well as

SIRT1 expression appear cell type-independent^[15-17]. However, since a considerable number of genes involved in cellular growth and senescence are direct or indirect targets of miR-34a^[18], it is unlikely that this miRNA can affect vascular aging only through SIRT1. Further studies are needed to shed more light on this aspect.

Further, we demonstrated that miR-34a is not only a senescence-associated miRNA, but it may influence inflammaging also by modulating other processes than senescence, such as inflammation, and in particular VSMCs SASP^[17]. Indeed, for the first time, we found that miR-34a could promote the expression of a subset of SASP molecules, specifically, the pro-inflammatory factors, IL1 β , IL6, IL8, BMP2, and MCP1, and the soluble adhesion molecule ICAM1. In contrast, no effect was observed on growth factors and their regulators IGFBP4/6, GRO/ α , and VEGF, and osteoprotegerin RNA levels. Accordingly, expression and secretion of pro-inflammatory SASP molecules have been found increased in VSMCs from aged animals as well as in replicative senescent VSMCs where the miR-34a is up-regulated^[17, 19, 20]. Furthermore, a very recent work from Fan and colleagues supports our findings leading to a pro-inflammatory role for miR-34a in vascular cells; indeed, they showed that this miRNA is involved in the flow-dependent regulation of endothelial inflammation by affecting VCAM1 and ICAM1 expression in ECs and consequently monocyte adhesion^[21].

The precise regulatory mechanisms responsible for SASP acquisition is currently unknown. In our *in vitro* experiments, the miR-34a-mediated transcriptional activation of pro-inflammatory SASP factors was not prevented by SIRT1 ectopic overexpression. Interestingly, very recently, Hayakawa *et al.* demonstrated that in x-radiation-induced senescent human fibroblasts, depletion of SIRT1 enhanced the transcriptional levels of SASP components (IL6 and IL8) through epigenetic gene regulation^[22]. The opposite was not observed in SIRT1 overexpression conditions^[22]. Yet, although, the SIRT1 activator resveratrol has been shown to reduce the secretion of IL1 β , MCP1 and IL6 by VSMCs^[19, 23], recently, Bollmann and colleagues demonstrated that resveratrol-dependent inhibition of cytokines expression occurs through the reduction of mRNA stability by the KH-type splicing regulatory protein (KSRP) activity and, is indeed, SIRT1-independent^[24]. Moreover, other factors rather than SIRT1 have been implicating in SASP regulation. Csiszar and co-workers, for instance, showed that the secretion of IL1 β , MCP1, TNF α and IL6 in aged VSMCs correlates with increased NF- κ B activation^[19]. Fan and colleagues showed that miR-34a affects ECs inflammation namely, VCAM1 and ICAM1 expression, through enhanced acetylation of the RelA/p65 subunit of NF- κ B and thereby

activation of NF- κ B signaling^[21]. They also observed that miR-34a-induced expression of these cell adhesion molecules is partially prevented by SIRT1^[21]. Since SIRT1 can regulate the acetylation of RelA/p65 subunit and thus decrease NF- κ B activation^[25], we speculate that in ECs miR-34a could influence NF- κ B signaling at least partially via SIRT1. Hence, unveiling the exact role of SIRT1 in SASP and whether miR-34a induces SASP factors expression in VSMCs by activating NF- κ B or other pathways calls for further investigations.

Taken together, all these experimental evidences indicate that miR-34a could be an important player in arterial inflammaging, since an age-dependent increase of its levels in vascular cells could enhance senescence and inflammation and thus cause arterial dysfunctions such as atherosclerosis. Accordingly, miR-34a expression has found augmented in human atherosclerotic lesions and in the aortas of an animal model of atherosclerosis^[26, 27].

Nonetheless, more studies are needed to deepen our knowledge on miR-34a involvement in arterial inflammaging, as this could be of extreme importance in order to develop an effective strategy beneficial for old people.

Conflicting interests

The authors declare that they have no conflicting interests.

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