miR-21 and miR-146a: The microRNAs of inflammaging and age-related diseases

miR-21 and miR-146a: the microRNAs of inflammaging and age-related diseases

Fabiola Olivieri [1,2], Francesco Prattichizzo [3], Angelica Giuliani [1], Giulia Matacchione [1], Maria Rita Rippo [1], Jacopo Sabbatinelli [1] and Massimiliano Bonafè [4]

1- Department of Clinical and Molecular Sciences, DISCLIMO, Università Politecnica delle Marche, Ancona, Italy

2- Center of Clinical Pathology and Innovative Therapy, IRCCS INRCA, Ancona, Italy

3- IRCCS MultiMedica, Milano, Italy

4- Department of Experimental, Diagnostic, and Specialty Medicine, University of Bologna, Bologna, Italy

Corresponding authors

Fabiola Olivieri, PhD

f.olivieri@univpm.it

Jacopo Sabbatinelli, MD, PhD

j.sabbatinelli@pm.univpm.it

Università Politecnica delle Marche

Department of Clinical and Molecular Sciences, DISCLIMO

Via Tronto 10/A, 60126 Ancona, Italy

Phone: +39.0712206243

Publisher version: https://doi.org/10.1016/j.arr.2021.101374

ABSTRACT

The first paper on "inflammaging" published in 2001 paved the way for a unifying theory on how and why aging turns out to be the main risk factor for the development of the most common age-related diseases (ARDs). The most exciting challenge on this topic was explaining how systemic inflammation steeps up with age and why it shows different rates among individuals of the same chronological age. The "epigenetic revolution" in the past twenty years conveyed that the assessment of the individual genetic make-up is not enough to depict the trajectories of age-related inflammation. Accordingly, others and we have been focusing on the role of non-coding RNA, *i.e.* microRNAs (miRNAs) in inflammaging. The results obtained in the latest 10 years underpinned the key role of a miRNAs subset that we have called inflammamiRs, owing to their ability to master (NF-κB)-driven inflammatory pathways. In this review, we will focus on two inflammamiRs, *i.e.* miR-21-5p and miR-146a-5p, which target a variety of molecules belonging to the NF-κB/NLRP3 pathways. The interplay between miR-146a-5p and IL-6 in the context of aging and ARDs will also be highlighted. We will also provide the most relevant evidence suggesting that circulating inflammamiRs, along with IL-6, can measure the degree of inflammaging.

Keywords. MicroRNA; miR-146a-5p; miR-21-5p; inflammaging; cell senescence; NF-κB.

1. Introduction

The first paper that minted the portmanteau word “inflammaging” – published about twenty years ago – laid the basis for a unifying theory on how and why aging is the major risk factor for the development of the most common age-related diseases (ARDs), spurring research into innovative inflammation-related ARD biomarkers (Franceschi et al., 2000a). The word “inflammaging” was coined to depict the building up of chronic, low-level, systemic inflammation occurring along human aging, and to highlight how inflammation boosts the susceptibility to chronic morbidity, disability, frailty, and premature death (Ferrucci and Fabbri, 2018; Franceschi et al., 2007; Furman et al., 2019; Piotrowicz and Gasowski, 2020). The most exciting challenge on this topic was to explain why inflammation undergoes activation with age. The results obtained so far emphasize that inflammation is fostered by a rife of external and internal stimulating factors that become more likely to show up with aging. The complex reshaping of the immune system with aging, defined by Roy Walford as immunosenescence (Pawelec, 2012; Pawelec et al., 2020; Walford, 1964) is likely to contribute to this framework, by promoting increased age-related persistence of self and non-self antigens, which in turn trigger long-lasting immune responses (Song et al., 2020a).

When the “epigenetic revolution” revealed that the genetic code is not enough to explain interindividual differences in aging rate, many efforts were devoted to disentangling the epigenetic factors involved in the modulation of the aging process. Consequently, others and we focused on short non-coding RNAs named microRNAs (miRNAs) (Carthew and Sontheimer, 2009)). Following this approach we identified a subset of miRNAs that master inflammatory pathways called inflammamiRs (Olivieri et al., 2013e). A number of these inflammamiRs target molecules belonging to the nuclear factor kappa B (NF-κB) and (NOD)-like receptor protein 3 (NLRP3) pathways. Lately, inflammamiRs have been identified as components the extracellular vesicle (EV) payload and were proposed as circulating biomarkers of ARDs (Rusanova et al., 2018). These data allowed us to put forth a nano-perspective of inflammaging (Mensa et al., 2020; Prattichizzo et al., 2017) in which we posited that circulating EV-associated miRNAs mediate, at least in part, the crosstalk between cellular senescence and aging at the systemic level (Olivieri et al., 2015a).

In this review, we will discuss how the discovery of the interaction between miRNAs and major inflammatory pathways improved our understanding of the mechanisms that modulate inflammaging, as well as ARD development and progression. We will focus on two prototypical inflammamiRs, i.e. miR-21-5p and miR-

146a-5p, that are able to target molecules belonging to the NF-κB/NLRP3 pathways, and on the interplay between miR-146a-5p and IL-6 in the context of aging and ARDs. Taking into account that miR-21-5p and miR-146a-5p are easily detectable in the bloodstream, as well as in other biological fluids, we will provide the most relevant and recent evidence about the role of circulating inflammamiRs, along with IL-6, as measures of the degree of inflammaging. Finally, a brief discussion of the therapeutic potential of miR-146a-5p and miR-21-5p in pro-inflammatory conditions and ARDs will be presented.

2. Inflammaging: basic concepts

Inflammaging is fostered by a number of external and internal stimulating factors: a plethora of non-self antigens can trigger the innate and adaptive immune responses across the entire human life span (De Martinis et al., 2005). Over the years, several basic mechanisms of inflammaging were identified. Among them, one of the most extensively investigated is the age-related accumulation of senescent cells (Coppe et al., 2008; Song et al., 2020b). Senescent cells release high amounts of soluble factors, collectively called as -senescence associated secretory phenotype, SASP, which encompasses a wealth of pro-inflammatory cytokines and chemokines, growth factors and extracellular matrix (ECM)-remodelling enzymes, thus contributing to a phenomenon defined as "senoinflammation" (Chung et al., 2019; Tchkonia et al., 2013**).** A wide range of molecular mechanisms converges on the acquisition of the senescent phenotype. One of the best characterized is the activation of a persistent DNA damage response (DDR), mainly triggered by genomic lesions and telomere attrition (Olivieri et al., 2015a; Rodier et al., 2009; Sulli et al., 2012). The activation of the unfolded protein response (UPR), a mechanism taking place in the endoplasmic reticulum (ER) and sensing protein biogenesis defects can also fuel inflammation (Gonzalez-Quiroz et al., 2020). In this latter case, the activation of the endoribonuclease inositol-requiring enzyme 1 alpha (IRE1α) can promote the degradation of a subset of mRNAs and microRNAs through a process known as regulated IRE1α-dependent decay of RNA (RIDD), thus impacting on various biological processes, including cell death and inflammation (Dufey et al., 2020). Finally, wide literature shows that the misplacement of nucleic acids into the cytoplasm is a powerful trigger of the senescent phenotype as well as of the cognate SASP (reviewed in Storci et al., 2018). Overall, senescent cells are characterized by the accumulation of DNA damage, unfolded proteins, and misplaced nucleic acids that can be sensed by specific intracellular receptors activating NF-κB (Storci et al., 2018). Noteworthy, SASP can

set off itself in neighboring cells, thereby creating a proinflammatory environment that propagates at the local and the systemic level (Fafian-Labora and O'Loghlen, 2020; Olivieri et al., 2015a).

The genetic or pharmacological clearance of senescent cells in animal models not only can extend the health span of prematurely and naturally aged mice, but also can taper chronic diseases and tissue inflammation (Prieto et al., 2020). The build in of senescent cells in aged animals and humans may be facilitated by immunosenescence (Burton and Stolzing, 2018). These data support the key role of senescent cells in fuelling inflammaging and hindering healthy aging (Franceschi et al., 2000a; Prieto et al., 2020). The induction of inflammation with aging has been claimed to depend on the excess of nutrients/energy that fuels an inflammatory process orchestrated by metabolic cells – defined as "metaflammation" (reviewed in Prattichizzo et al., 2018a). The increased burden of altered molecules deriving from damaged and/or dead cells and organelles, defined as "garb-aging", has been regarded to share in this scenario (Franceschi et al., 2017). Regardless of the specific name by which these forms of inflammation have been called, they are all characterized by the persistence of a chronic, low grade, and systemic inflammatory status, i.e. inflammaging (Franceschi et al., 2000a; Franceschi and Cossarizza, 1995). The existence of a huge variety of stimuli able to fuel inflammaging is explained by the observation that a reduced number of ancestral pathways, including NF-κB/NLRP3, can be activated by a variety of internal and external stimuli and converge on cytokines and IFN gene transcription (Gritsenko et al., 2020; Meyers and Zhu, 2020; Ren et al., 2014; Sebastian-Valverde and Pasinetti, 2020; Storci et al., 2020; Teodori et al., 2019).

The activation of these signalling systems constitutes the main arm of innate immunity, on the other side, their chronic activation, as occurs in senescent cells, becomes detrimental for cell/organism homeostasis, making aged people more susceptible to ARDs (Meyers and Zhu, 2020; Youm et al., 2013; Yu et al., 2015).

Another challenge found in studies on inflammaging was to explain why it proceeds at different rates among individuals. Indeed, the concept that individuals do not age at the same pace entails the notion of biological age which differs from chronological age (Hamczyk et al., 2020). Biological age results from the complex interaction between the genetic make-up and the epigenetic modifications induced by environmental factors: each individual is characterized by a unique combination of these intrinsic and extrinsic factors (Rose et al., 2010). Therefore, subjects sharing the same genetic profile, like monozygotic twins, or the same chronological

age, like peers, can differ in biological age as well as in the degree of inflammaging owing to epigenetic factors and mechanisms (Franceschi and Bonafe, 2003).

3. Key roles of NF-κB and NLRP3 in inflammaging

NF-κB signalling is the master regulator of innate immunity and the key hub of danger signals response (Salminen et al., 2008).

Briefly, NF-κB family is set up by inducible homo or hetero-dimeric transcription factors, including five members, i.e. Rel (c-Rel), RelA (p65), RelB, NF-κB1 (inactive precursor p105, mature protein p50), and NF-κB2 (inactive precursor p100, mature protein p52), present in the cytoplasm as inactive molecular complexes (Liang et al., 2004). NF-κB activation involves IκB kinase (IKK), a complex of two catalytic subunits (α and β), and a regulatory protein known as NF-κB Essential Modifier (NEMO) (DiDonato et al., 1997). When cells are stimulated, NF-κB dimers translocate from the cytoplasm to the nucleus and activate a number of responsive gene promoters containing highly divergent κB-site sequences (Mulero et al., 2019). The activation of NF-κB is mainly promoted by two different signalings, named classical (or canonical) and alternative (or non-canonical) activation (Hayden and Ghosh, 2004) (summarized in **Figure 1**).

The canonical NF-κB signalling is rapidly and transiently activated by a number of ligands (PAMPS/DAMPS), independently of protein synthesis (Beinke and Ley, 2004). The non-canonical signalling is slowly and persistently activated by TNF superfamily members, such as macrophage colony-stimulating factor (M-CSF), a growth factor that mediates the differentiation and proliferation of macrophages, and several RNA viruses (Sun, 2017).

Importantly, though NF-κB1 (p105) is constitutively processed to p50 following intense stimulation with agonists, such as TNF-alpha and lipopolysaccharide (LPS), NF-κB1 is completely degraded by the proteasome, suggesting that this subunit can play an important regulatory role shifting NF-κB activity from “on” to “off” (Cartwright et al., 2016). Non-canonical NF-κB2 is activated through proteasomal activation of p100 in response to stimuli only partly overlapping with those activating NF-κB1. Increasing evidence suggest that NF-κB1 mediates transient responses to a plethora of stimuli, whereas NF-κB2 modulates prolonged pro-inflammatory effects (Wirasinha et al., 2021).

The initial *in vivo* investigations showed that NF-κB activity was upregulated in both naturally aged mice and multiple progeroid mouse, and that p65 deletion and/or treatment with IKK pharmacological inhibitors delayed the onset of age-related features and cellular senescence (Osorio et al., 2012; Tilstra et al., 2012). Subsequent studies focused on the specific NF-κB1 subunit deletion (Nfkb1 -/- mice), showing surprisingly that the loss of NF-κB1 can accelerate the aging process. Nfkb1-/- mice were characterized by low-grade chronic inflammation and premature aging, appearing as a model of genetically enhanced NF-κB activity. This apparent paradox can be explained by considering that the first p65 knockout mouse models carried only one inactivated p65 allele (Osorio et al., 2012; Tilstra et al., 2012). Recent studies confirmed that the complete knockout of p65 in mice promotes osteoarthritis (OA), while p65 haplo-insufficiency protects against OA, suggesting that the partial down-regulation of canonical NF-κB activity can exert beneficial effects (Kobayashi et al., 2016).

Nfkb1-/- mice showed higher incidence of age-related phenotypes, an increased burden of senescent cells, and a decrease in overall lifespan compared to Nfkb1+/+ (Bernal et al., 2014). These features appeared to be mediated by a Cox-2-mediated stabilization of ROS-induced DNA damages at telomeres (Jurk et al., 2014). These observations were recently confirmed showing that Nfkb1-/- mice develop gastric mucosal disease following the administration of tamoxifen, a process associated with an exaggerated DNA damage response at the gastric epithelium level (Burkitt et al., 2017), as well as invasive gastric cancer with high levels of pro-inflammatory cytokines, such as IL-6 and TNFα (Low et al., 2020). Moreover, during aging Nfkb1-/- mice show an early onset of memory loss, combined with enhanced neuroinflammation and increased burden of senescent cells in the hippocampus and cerebellum, strongly supporting the hypothesis that chronic inflammation is a causal factor in the cognitive decline observed during aging (Fielder et al., 2020).

Overall, these data suggest that the complete loss of NF-κB1 activity (as in Nfkb1−/− mice) leads to early animal aging associated with reduced apoptosis and increased cellular senescence. Studies on cellular models highlighted that the loss of Nfkb1 is not commensurate with inhibition of NF-κB activity when it is analysed as total activity, as Nfkb1−/− cells retain significant levels of DNA bound NF-κB (Hoffmann et al., 2003).

NF-κB2 has been involved in the pathogenesis of several human diseases characterized by age-related persistent inflammatory condition, as suggested by the findings revealing that the RelB/NF-κB2 pathway

157 regulates T cell migration to autoimmune targets through TGFβ/TGFβR-dependent regulation of
158 CXCL12/CXCR4 signalling (Kurosawa et al., 2017).
159 Overall, these findings suggest that the increase in NF-κB DNA-binding activity with age is related to p52/NF-
160 κB2 rather than p50/NF-Kb1, and that the switch in the DNA binding from DNA-bound p50/NF-κB1 to
161 p52/NF-κB2 is a prominent feature of physiological aging.
162 A large number of stimuli can promote a very different transcriptional profile and physiological outcomes
163 according to the specific trigger and the cell type involved. The NF-κB "barcode hypothesis" posits that cellular
164 network dynamics generate NF-κB signal-specific post-translational modifications, mediating specific gene
165 expression pattern activation (Peng et al., 2016). These observations are relevant to explain the effects of the
166 complex epigenetic modulation of the NF-κB signalling on aging.
167 NF-κB activation promotes the hyper-expression of target genes involved in pro-inflammatory/stress-like
168 responses, including pro-IL-1β and pro-IL-18. The proteolytic activation of these cytokines requires the
169 activation of the NLRP3 (also known as cryopyrin or NALP3) inflammasome (Jo et al., 2016). TLR-NF-κB
170 pathway activation promotes the upregulation of NLRP3, which responds to a second signal consisting in
171 various pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns
172 (DAMPs), as well as multiple molecular and cellular events that finally activate the functional NLRP3
173 inflammasome (reviewed in Kelley et al., 2019). The activation of the NLRP3 inflammasome consists in the
174 assembly of the multi-protein complex NLRP3-ASC-CARD that induce the splicing of pro-IL-1β and pro-IL-
175 18 precursors into mature active forms (Jo et al., 2016; Strowig et al., 2012). NLRP3 also interacts with NOD2-
176 activating NF-κB and MAPK pathways, thus leading to a positive loop inducing that results in the transcription
177 of pro-inflammatory genes (Murray, 2009).
178 Overall, an efficient response to a variety of antigens strictly depends on two types of signals, i.e. a priming
179 signal that activates NF-κB trough membrane-bond receptors (TLRs, RAGEs, TNFR, RIG, NOD) and a second
180 signal that triggers the assembly of the NLRP3 complex.
181 When transiently activated, NF-κB (mainly the canonical pathway) and NLRP3 orchestrate a timely
182 coordinated inflammatory response, whereas when their activation is prolonged over time (mainly non
183 canonical NF-κB pathway) they drive cells into a growth arrest associated with enhanced p21 (cyclin-
184 dependent kinase inhibitor 1A) protein expression, fostering the acquisition of cellular senescence phenotype

(Penzo et al., 2009). Therefore, NF-κB/NLRP3, the major signalling pathway inducing an inflammatory response, also triggers SASP (Salminen et al., 2012), representing a link between innate immunity and cellular senescence. Macrophages are the major source of IL-1β and IL-18 through the activation of NF-κB/NLRP3 (Man and Kanneganti, 2015). Thus, their prominent role in inflammaging, a phenomenon that we described for the first time as "macroph-aging" has been envisaged (Franceschi et al., 2000b; Prattichizzo et al., 2016a; Sebastian et al., 2005).

Indeed, the NLRP3 inflammasome is over-activated in aged individuals, in whom it leads to a hyper-response of classically activated macrophages and subsequent increases in IL-1β production (Lara et al., 2020).

Consistently with the hypothesis that NLRP3 activation fuels inflammaging and promotes ARDs, an improvement of glycemic control and bone loss was observed in mice lacking the activity of the NLRP3 inflammasome-mediated caspase-1 (Youm et al., 2013). Interestingly, the abrogation of NLRP3 inflammasome was also correlated with reduced rate of age-related thymic atrophy, thus increasing T cell repertoire diversity (Youm et al., 2012). These data demonstrate that preventing the inflammasome-dependent caspase-1 activation prevents immunosenescence by increasing thymic lymphopoiesis. These observations were recently corroborated by observations in NLRP3 inflammasome knockdown mice models, that can be summarized as follow: 1) the promotion of healthy life-span, 2) the improvement of metabolic health and lifespan in obese mice, 3) the prevention of cardiac dysfunction in aged male mice (Canadas-Lozano et al., 2020; Marin-Aguilar et al., 2020a; Marin-Aguilar et al., 2020b).

To avoid an exacerbated inflammatory response, some inhibitors can act on NF-κB and NLRP3 pathways. One of the best characterized inhibitors of both pathways is A20, also named TNF inducible protein 3 (TNFAIP3), a cytosolic inhibitor of NLRP3 inflammasome and of the NF-κB and MAPK signalling cascades (Vande Walle et al., 2014; Xue et al., 2019b). A20 limits the magnitude of inflammatory response triggered by different stimuli and prevents spontaneous IL-1β secretion. Accordingly, A20-deficient mice show excessive cytokine secretion and caspase-1 processing in myeloid cells and exhibit enhanced arthritis pathology, which was dependent on the NLRP3 inflammasome (Vande Walle et al., 2014), and spontaneously developed a systemic inflammatory syndrome that culminated in premature death (Lee et al., 2000). Overall, the increasing evidence obtained by *in vitro* and *in vivo* models confirmed that NF-κB and NLRP3 are the key mediators of

inflammaging. Several inflammamiRNAs that can efficiently modulate these pathways are the best candidates to serve as innovative functional biomarkers of human aging and ARDs.

4. InflammamiRNAs that regulate NF-κB and NLRP3: focus on miR-21-5p and miR-146a

Several miRNAs have been proposed and validated for their ability to target molecules belonging to the NF-κB and NLRP pathways and take part in positive and negative feedback loops, along with different cell types, timings and experimental conditions (reviewed in Poli et al., 2020). Here, we focus on the role of the two inflammamiRs, namely miR-21-5p and miR-146a, both involved in the modulation of NF-κB and NLRP pathways. Notably, i) the presence of NF-κB-binding sites in both miR-21-5p and miR-146a gene transcriptional elements shows that these miRNAs are directly regulated by this transcription factor (Shin et al., 2011; Taganov et al., 2006); ii) miR-21-5p ad miR-146a levels significantly change both in tissues and in the bloodstream during aging and are associated with a variety of inflammatory conditions and ARDs (Olivieri et al., 2013c; Olivieri et al., 2015b; Olivieri et al., 2012).

4.1 MiR-21-5p

MiR-21 gene is abundantly expressed in multiple types of mammalian cells, and it is located on chromosome 17q23.2 (55273409–55273480) within the coding gene for vacuole membrane protein-1 (VMP1) gene, also known as transmembrane protein 49 (TMEM49). Despite pri-miR-21 and TMEM49 are overlapping genes in the same direction of transcription, pri-miR-21 is independently transcribed by its own promoter region. Thus, miR-21 is a unique miRNA capable of being regulated by two independent promoters (Ribas et al., 2012). MiR-21 is induced by a number of pro-inflammatory molecules, such as PAMPs and DAMPs able to activate NF-κB and NLRP3 (Fujita et al., 2008). MiR-21 orchestrates the fine-tuning of the inflammatory response through direct and indirect activities on NF-κB and NLRP3 pathways in a context-dependent manner. *In vitro* and *in vivo* studies on animal models confirmed the essential role played by miR-21 in regulating this inflammatory switch, both promoting or inhibiting NF-κB/NLRP3 pathways. For all these reasons, miR-21 can be considered as a miRNA able to modulate the “switch on/off” of inflammation at appropriate times. When the *in vivo* activity of miR-21 was examined in healthy mouse liver using array profiling and polysome analysis, it turned out that miR-21 is functionally limited under normal physiological conditions compared to

other miRNAs, probably because the interaction between miR-21 and polysome-bound mRNAs is prevented (Androsavich et al., 2012). In pathological conditions, when the mRNAs targeted by miR-21 are transcribed at increased rate, miR-21 transcription significantly increases. Multiple evidence confirmed that miR-21-5p can target transcripts of tumour suppressor genes, such as phosphatase and tensin homolog (PTEN) and programmed cell death 4 (PDCD4) (Matsuhashi et al., 2019). Importantly, PDCD4 takes part in the activation of NF-κB and enhances the production of IL-6, thereby limiting the production of the anti-inflammatory cytokine IL-10 (Sheedy, 2015; Sheedy et al., 2010). Therefore, PDCD4 acts as a molecular switch that shifts the balance towards a pro-inflammatory microenvironment. In this framework, by opposing the effects of PDCD4, miR-21 attenuates persistent inflammation by restraining NF-κB activation and IL-6 production and augmenting the production of IL-10.

MiR-21 is among the most abundant and highly conserved recognized miRNAs, and it is highly expressed in monocytes/macrophages (Jenike and Halushka, 2021). Alterations in the local or systemic expression of miR-21 have been reported in a large range of cardiovascular diseases (Kura et al., 2020). On one side, the absence of miR-21 in hematopoietic cells enhances the progression of atherosclerosis by promoting the formation of foam cells (Canfran-Duque et al., 2017). On the other side, in mouse models of myocardial ischemia/reperfusion injury, miR-21 expression is reduced in infarcted areas, while it is up-regulated in borderline areas (Dong et al., 2009; Pan et al., 2018). Of note, the decreased expression of miR-21 in tissues persists for two weeks, while the restoration of its expression abrogates cardiomyocyte apoptosis and pro-fibrotic responses and results in reduced infarct size and improved cardiac remodelling (Gu et al., 2015). Worth mentioning, miR-21–null mice had lower platelet and leukocyte counts, but higher megakaryocyte number in the bone marrow, as well as the pharmacological inhibition of miR-21 reduced the platelet release of TGF-β1, overall restraining fibrosis (Barwari et al., 2018).

Nevertheless, miR-21 acts as a positive regulator of the NF-κB/NLRP3 pathways by targeting its inhibitor A20 (Xue et al., 2019b). In murine models of septic shock or periodontitis, miR-21 promotes the activation of NF-κB and the NLRP3 inflammasome (Xue et al., 2019b; Zhou et al., 2018b). Similarly, in a mouse model of diabetic nephropathy, miR-21 loaded into macrophage-derived EVs regulates pyroptosis-mediated podocyte injury, by activating NF-κB/NLRP3 (Ding et al., 2020b).

Beyond its regulatory role, miR-21 has been also demonstrated to act as an endogenous ligand of TLR8, a receptor expressed on intracellular vesicular membranes and commonly involved in the recognition of GU-rich single stranded RNA (Heil et al., 2004). TLR8, in turn, triggers both ERK (Zhang et al., 2018b) and NF-κB (Fabbri et al., 2012) activation to induce the secretion of inflammatory mediators.

Of note, nanoparticle-mediated delivery of miR-21 mimicking molecules to cardiac macrophages relieves post-myocardial infarction remodelling and heart failure (Bejerano et al., 2018). Furthermore, long-term inhibition of miR-21 favourably affects cardiac fibrosis supporting the idea of a biphasic, context- and time-dependent effect of miR-21 on inflammatory pathways (Thum et al., 2008).

A similar framework applies also to another prototypical ARD, *i.e.* T2DM (Prattichizzo et al., 2016b). Indeed, initial findings showed a decreased expression of miR-21 in diabetic patients (Olivieri et al., 2015b; Zampetaki et al., 2010), albeit also the opposite has been reported (La Sala et al., 2019). Noteworthy, miR-21 loaded CD31-positive circulating EVs increase in diabetic subjects and further augmented in those patients with cardiovascular complications (Prattichizzo et al., 2021a). These data suggest that miR-21 shuttled in specific vesicle might hold superior diagnostic potential for ARDs sensing when compared to its whole plasma counterpart (Prattichizzo et al., 2021a; Prattichizzo et al., 2019). Mechanistically, metabolic alterations are known to induce pro-inflammatory responses (Prattichizzo et al., 2020); interestingly glucose induces an increase of miR-21 expression *in vitro* models thus suggesting its involvement in the above-mentioned responses (La Sala et al., 2018). Accordingly, T2DM patients with longer diabetes duration are characterized by a progressive, complex inflammatory remodelling and the development of specific vascular complications, many of which modulate miR-21 levels (Prattichizzo et al., 2016b).

A further layer of complexity into the interpretation of miRNA biological roles comes from bioinformatic analyses of small RNA-seq data revealing the existence of multiple miRNA isoforms, i.e isomiRs, that can be generated from the processing of each miRNA precursor. IsomiRs are characterized by the addition or deletion of one or more nucleotides at the 5' and/or 3' ends of the canonical miRNA sequence (Telonis et al., 2015). In addition, miRNAs can undergo post-transcriptional sequence modifications resulting in non-templated adenylation or uridylation at the 3' end (Vitsios et al., 2017). Even if the relationships between these 3' modifications and the targetome depending from the seed sequence are not so clear, increasing evidence suggested that these modifications can affect the stability of the RNA sequence (van der Kwast et al., 2020),

and can affect miRNA loading into EVs (Koppers-Lalic et al., 2014). Notably, isomiR analysis unravelled specific gender- and population-based patterns of expression in a variety of diseases (Loher et al., 2014). Two mature biologically active miR-21 isomiRs are highly represented, with the cell-type specific ratio of the two isoforms. The longer isoform, consisting of a 23-nt sequence, derives from the DICER1-mediated cleavage of pre-miR-21, while the shorter isoform, corresponding to the 22-nt miRbase sequence, derives from a tailing and trimming process of the former (Boele et al., 2014). This apparently redundant network, which allows for a tight regulation of miR-21 degradation, plays a key role on the miRNA-based regulation of the p53 pathway, providing an exploitable target in the treatment of human proliferative disorders (Shukla et al., 2019).

We recently performed a comprehensive analysis of miRNAs and isomiRs profile in HUVECs undergoing replicative senescence in presence of metformin treatment, a glucose lowering drug being tested as a potential aging-modifying compound (Prattichizzo et al., 2018b). Almost 40% of the total miRNAs pool was composed by non-canonical sequences, and metformin significantly affected the relative abundance of a number of isomiRs, including the non-canonical miR-21 forms, supporting the notion that the relative proportion of isomiRs might also mark cellular aging and the relative interventions (Giuliani et al., 2020).

4.2 MiR-146a

The miR-146 family consists of 2 members, *i.e.* miR-146a and miR-146b, found on human chromosomes 5 and 10, respectively. *MIR146A* gene is found within a larger long noncoding RNA host gene – MIR3142HG (chromosome 5q33.3) – while MIR146B is found in an intergenic region of human chromosome 10 (10q24.32). The miRBase database provides evidence for the mature -5p strand to be the bioactive "guide strand" and the mature -3p strand to be the "passenger strand" for both miR-146a and miR-146b. Therefore, the mention of miR-146a and miR-146b is usually referred to the -5p strands. The mature sequences for miR-146a and miR-146b are highly conserved across species. Notably, the two miRNAs differ only by two nucleotides on the 3′-end of the mature strand, not within the seed region. However, despite this similarity, the two miRNAs are not functionally redundant *in vivo*.

MiR-146a was the first to be identified as NF-κB-dependent miRNA, being up regulated in response to various immune mediators, such as LPS, IL-1β, and TNF-α (Taganov et al., 2006). In turn, miR-146a down-regulates relevant proteins which share in the canonical NF-κB pathway, such as Toll like receptor (TLR-)4, myeloid

differentiation primary response gene 88 (MyD88), Interleukin-1 receptor-associated kinase 1 (IRAK1) and TNF receptor associated factor (TRAF6) (d'Adhemar et al., 2014; Olivieri et al., 2013b; Taganov et al., 2006). Notably, miR-146a also targets RelB, a key factor of the non-canonical NF-κB pathway, in macrophages (Etzrodt et al., 2012) and lung fibroblasts (McMillan et al., 2013). Hence, its ability to modulate both NF-κB pathways makes it an eligible functional biomarker of inflammaging.

IRAK1 is a miR-146a target in different cells and experimental settings, whereas the relationship between miR-146a and TRAF6 is likely to be context dependent (Bertolet et al., 2019). There is evidence that TRAF6 activates Akt and mTOR, thus explaining how these pathways are impacted by miR-146a (Yang et al., 2009). TRAF6 itself localizes to the mitochondria during TLR activation, a process that is required for activation of inflammation and ROS production by macrophages (West et al., 2011)**.** Interestingly, miR-146a has been showed to be overexpressed within mitochondria of ageing human endothelial cells where i) it down-regulates Bcl-2 family members, ii) induces the opening of the permeability transition pore and the activation of caspases 1 and 3, iii) affects sensitivity to apoptosis and autophagy, thus modulating cell function and inflammaging (Giuliani et al., 2018).

Defining the cells in which miR-146a exerts its action is fundamental to deepen our knowledge on the role played by this miRNA in aging and ARDs. I*n vitro* models convey that deficiency of miR-146a induces deregulation of TRAF6 in synovial fibroblasts, leading to increased proliferation, metabolic shift towards glycolysis, enhanced ability of synovial fibroblasts to support the generation of osteoclasts (Saferding et al., 2017).

MiR-146a overexpression can be induced both by pro-inflammatory conditions, *i.e.* treatment with LPS, and replicative or stress-induced senescence, reinforcing the hypothesis of the key role of miR-146a in inflammation and senescence (Olivieri et al., 2013b; Sugimoto et al., 2016).

Mice with targeted miR-146a deletion represent one of the first genetic animal models with NF-κB-driven low-grade inflammation that spontaneously develops with aging. NF-κB activation increases in miR-146a$^{-/-}$ mice, suggesting that miR-146a can negatively modulate inflammation by targeting key molecules in both NF-κB activation arms. Importantly, young miR-146a–null mice did not develop any disease, whereas exhibited signs of multiorgan inflammation and immunoproliferative disease at 6–8 months (Boldin et al., 2011; Zhao et al., 2011). These results provide a molecular connection between chronic inflammation, the development of

bone marrow failure and myeloproliferative neoplasms (Zhao et al., 2013). In animal models, the decline of TRAF6 gene expression in miR-146a$^{-/-}$ mice rescued aberrant myeloproliferation and autoimmunity phenotypes (Magilnick et al., 2017). Recently, it was demonstrated that the administration of a DNA oligonucleotide-conjugated miR-146a mimic binds Toll-like receptor 9 prevents excessive NF-κB activation in myeloid cells, alleviating myeloproliferation and mice hypersensitivity to bacterial challenge (Su et al., 2020). Notably, miR-146a was identified as an essential epigenetic switch that controls Wnt signalling-dependent bone homeostasis during aging (Saferding et al., 2020). MiR-146a deficient mice develop early onset and progression of osteoarthritis (Guan et al., 2018), whereas in humans, the levels of miR-146a are increased in patients suffering of bone fractures (Saferding et al., 2020).

Further, miR-146a$^{-/-}$ mice on high-fat diet showed exaggerated weight gain, increased adiposity, hepatosteatosis, and dysregulated blood glucose levels compared to wild-type controls (Runtsch et al., 2019). These observations suggest that, during inflammation, miR-146a represses TRAF6 not only to control inflammatory gene expression, but also to limit the switch from oxidative phosphorylation to glycolytic metabolism (Kelly and O'Neill, 2015). *In vivo* functional evidence suggested an anti-inflammatory role of miR-146a in the pathogenesis of diabetic nephropathy (DN) (Bhatt et al., 2016): long-term hyperglycemia induces miR-146a downregulation in the sciatic nerve of DN rats causing a loss of NF-κB inhibition, tissue damage, TNF-α, and IL-1β release (Feng et al., 2018). Importantly, in mice model of DN the treatment with miR-146a mimics as able to improve neurological function (Liu et al., 2017). Regarding a potential role of miR-146a in modulating atherosclerosis, it was suggested a link between miR-146a and apolipoprotein E (ApoE): systemic delivery of miR-146a mimetic can attenuate macrophage activation and atherosclerosis also in the absence of plasma lipid reduction (Li et al., 2015a).

Finally, miR-146a-deficient mice have hyper-reactive and pro-inflammatory circulating neutrophils that are more prone to form neutrophil extracellular traps (NETs), regardless of the stimulus (Arroyo et al., 2020). NETs, which consist of cell-free DNA (cfDNA), histones, NE (neutrophil elastase), and myeloperoxidase, can triggers the coagulation and inflammation underlying cardiovascular complications; in this framework miR-146a modulation could be of clinical relevance in cardiovascular diseases (Arroyo et al., 2020).

Notably, it as demonstrated that miR-146a can modulate also the NLRP3 inflammasome related factors, such as its downstream inflammatory factors and its upstream protein chloride intracellular channel protein 4 (CLIC4) expression both *in vitro* and in animal models (Zhang et al., 2018a).

Overall, the take-home message of the last ten years of studies on the relationship between miR-146a and inflammation is that miR-146a exerts a number of beneficial effects on healthy aging, via a fine-tuning of canonical and non-canonical NF-κB pathways **(Figure 1)**. It is therefore not surprising that the fates of miR-146a and IL-6 seem to be intertwined, as it is detailed below.

4.3 MiR-146a and IL-6 regulation

Circulating levels of IL-6, "the cytokine for gerontologists" (Ershler, 1993), increase significantly with aging and are associated with hampered chance to achieve healthy longevity and successful aging (Albani et al., 2009; Bonafè et al., 2001; Bonafe et al., 2001; Ferrucci et al., 2005; Kim et al., 2012; Leng et al., 2005; Lin et al., 2014; Maggio et al., 2006; Stenholm et al., 2011). Since high IL-6 circulating levels are associated with increased mortality risk in frail and community-dwelling elderly individuals (Giovannini et al., 2011), whilst low IL-6 serum levels are associated with successful aging (Akbaraly et al., 2013), IL-6 can be considered as the most clinically relevant circulating biomarker of inflammaging (Bonafe et al., 2001; Ferrucci and Fabbri, 2018). The Cardiovascular Health Study, the InCHIANTI study, and the PolSenior study showed that both IL-6 and CRP levels are good predictors of physical and cognitive performance and the risk of mortality in the entire elderly population and in successfully aging individuals (Puzianowska-Kuznicka et al., 2016). Genetic variability at the IL-6 locus, as well as in other cytokines have been positively or negatively associated with longevity (Bonafe et al., 2001; Capri et al., 2014; Franceschi et al., 2020; Lin et al., 2014; Maggio et al., 2006; Newman et al., 2010; Rosa et al., 2019; Stenholm et al., 2011).

IL-6 exerts its activity through binding to the IL-6 receptor (IL-6R), which consists of two types of subunits, IL-6Rα (gp80 or CD126), and IL-6Rβ (gp130 or CD130). The soluble IL-6R (sIL-6R), which is cleaved from the cell membrane, can still bind IL-6 (Tanaka and Kishimoto, 2014). The paradigm of IL-6 signal transduction via the membrane bound IL-6R is called "classic signalling", whereas when IL-6 signal is activated by the link with sIL-6R, it is referred to as "trans-signalling" (Garbers et al., 2012). The sIL-6R binds IL-6 with

comparable affinity as the membrane bound form and mediates gp130 activation. Notably, the regenerative or anti-inflammatory activities of IL-6 are mediated by classic signaling whereas pro-inflammatory responses of IL-6 are rather mediated by trans-signaling (Rabe et al., 2008; Scheller et al., 2011). A number of studies performed about thirty years ago were focused on the complex regulatory mechanisms of IL-6 gene expression induced by different transcriptional factors, such as NF-κB and AP-1, and their synergistic combinations (Libermann and Baltimore, 1990; Son et al., 2008; Wisdom, 1999). NF-IL6 is another nuclear factor that specifically binds to the promoter of IL-6 gene; this nuclear factor has a high degree of homology to a liver- and adipose tissue-specific transcriptional factor named CCAAT/enhancer binding protein beta (C/EBP-β) (Akira et al., 1990). Notably, the involvement NF-IL6/C/EBP-β transcription factor in senescence-associated enhancement of cytokine expression was described (Kuilman et al., 2008). C/EBP-β can enhance the binding activity of p65 to IL-1β promoter, thus suggesting a complex interaction between NF-κB/NLRP3 inflammasome and IL-6 (Ma et al., 2018). Notably, NF-IL6 and C/EBP-β recognize the same nucleotide sequence but exhibit distinct patterns of expression. NF-IL6 mRNA is normally not expressed, but it can be induced by the stimulation with exogenous antigens, i.e. LPS, and also by endogenous molecules, i.e. IL-1, synthesized after NF-κB/NLRP3 activation. Interestingly, NF-IL6 was shown to bind the regulatory regions of various acute phase protein genes and several other cytokine genes such as TNF, IL-8 and G-CSF, implying that NF-IL6 has a role in the regulation not only of the IL-6 gene but also of several other genes involved in acute-phase reaction, inflammation and hemopoiesis (Natsuka et al., 1992). Three isoforms of NF-IL6 (C/EBPL) are generated from a single mRNA, including LIP (liver-enriched inhibitory protein) and LAP (liver-enriched activator protein). C/EBP homologous protein (CHOP), a member of the C/EBP family of transcription factors, by forming heterodimers with LIP, can increase LAP activity and transactivational ability (Zinszner et al., 1998). Overall, as described above, IL-6 transcription is regulated by a number of nuclear factors that can act in independent or synergistic way, especially in the perpetuation of IL-6 increased release for a long time. The recent research is aimed to disentangle additional layers in the complex mechanisms of IL-6 epigenetic regulation, analysing DNA methylation and non-coding RNAs modulation (Servais et al., 2019).

The transcription of both miR-146a and IL-1β/IL-6 is under the control of NF-κB, even if the two molecules –IL-6 and miR-146a – play opposite roles in the inflammatory process. Therefore, one would expect a

proportional increase in IL-6 and miR-146 synthesis, in order to restrain the excessive IL-6 increase. As above reported and discussed, although miR-146a does target neither IL-6 mRNA nor its receptors, a number of *in vitro* and *in vivo* studies highlighted a negative relationship between miR-146a and IL-6 levels. These evidence ere confirmed by functional studies demonstrating that miR-146a mimics decrease, while miR-146a inhibitors increase the expression of IL-6 in LPS-stimulated macrophages (He et al., 2014; Luly et al., 2019) and activated endothelial cells (Pfeiffer et al., 2017).

When the relationship between miR-146a and IL-6 was analysed in the framework of the aging process, an age-related inbalance of miR-146a/IL-6 axis was observed (Jiang et al., 2012; Olivieri et al., 2013d). Aged miR-146a−/− mice display elevated serum levels of IL-6, thus supporting the functional link between miR-146a and IL-6 levels (Boldin et al., 2011). However, miR-146a expression is modulated at a different extent in response to LPS stimulation in macrophages of young and aged mice (Jiang et al., 2012). Despite macrophages of aged mice release an increased amount of IL-6 compared with younger ones, miR-146a level increases significantly in a cytokine dose-dependent manner only in macrophages of young mice (Jiang et al., 2012). The same results can be observed in young and senescent endothelial cells stimulated with LPS (Olivieri et al., 2013d; Olivieri et al., 2014). These results point out the imbalance between miR-146a and IL-6 production in senescent cells, suggesting that nuclear factors different from NF-κB could promote IL-6 transcription. For example, following the activation of the unfolded protein response (UPR) CHOP plays an important role in IL-6 production without binding to its promoter, probably by trapping protein which would otherwise inhibit IL-6 transcription (Hattori et al., 2003).

Concerning circulating miR-146a, its levels decline in old wild-type mice (Grants et al., 2020), as well as in healthy aged people (Mensà et al., 2019), whereas in the same conditions IL-6 levels are increased**.** In patients affected by T2DM this loop – low levels of miR-146a and increased IL-6 levels – is more evident. Notably, COVID-19 patients showed increased IL-6 and reduced miR-146a-5p circulating levels compared to healthy age-matched subjects, pointing again at the involvement of imbalanced IL-6/miR-146a-5p axis in the cytokine storm observed in SARS-CoV-2 infection (Sabbatinelli et al., 2021). Noteworthy, a similar scenario was reported in the context of sepsis (Benz et al., 2016).

We can hypothesize that, in physiological conditions, the miR-146a/IL-6 axis is under the control of NF-κB, thus maintaining a functional balancing, whereas in the presence of relevant pro-inflammatory stimuli, IL-6

levels dramatically increase and miR-146a levels decline. Importantly, the age-related decline of miR-146a in healthy subjects is particularly evident in males (Mensà et al., 2019), strongly supporting the increased susceptibility of men to the detrimental effects of inflammaging. Notably, differences in immune functions can contribute to health- and life-span disparities between sexes (Jaillon et al., 2019; Marquez et al., 2020). Epigenomic signature of immunosenescence includes the decline of naïve T cell and the increase in monocyte and cytotoxic cell functions (de Candia et al., 2021; Fulop et al., 2018). Notably, these features occur to a higher extent in men than in women, suggesting that males are characterized by higher pro-inflammatory activity and lower adaptive immune system activity (Marquez et al., 2020).

Overall, we can conclude that a weak age-related increase of circulating IL-6 levels can be observed in healthy aging in association with declined miR-146a circulating levels. On the contrary, aged subjects affected by ARDs showed a stronger age-related IL-6 increase and miR-146a decrease, suggesting that in these conditions miR-146a levels are not sufficient to restrain inflammatory status.

5. miR-21 and miR-146a as biomarkers of age-related diseases

The identification of biomarkers of healthy/unhealthy aging trajectories is an urgent need, which could contribute to an early diagnosis of ARDs and improve the monitoring of novel interventions (Guerville et al., 2020; Newman et al., 2016). We are among the first researchers hightlighting significant changes of miR-21-5p and miR-146a circulating levels in healthy subjects of different ages, thus suggesting that some inflammamiRs can contribute to trak an healthy aging trajectory (Olivieri et al., 2017; Olivieri et al., 2013d; Olivieri et al., 2012). Intriguingly, derangements from the levels observed in the healthy aging trajectory were observed in patients affected by different ARDs, suggesting that miR-21-5p and miR-146a circulating levels could be useful biomarkers of ARDs and their complications (Olivieri et al., 2017; Olivieri et al., 2013e; Olivieri et al., 2012). Other studies on the same topic identifyed the age-related deregulation of a number of circulating miRNAs (Balzano et al., 2017; Noren Hooten et al., 2013). For instance, when miR-21 and miR-146a serum levels were measured in a sample of sprint-trained male athletes and associated with physical performance, significant correlation with age were observed as well as significant associations with the 10-year decline of physical performance (Kangas et al., 2017).

When miRNA expression signatures were analysed in Treg lymphocytes from patients with autoimmune diseases, such as rheumatoid arthritis, the analysis allowed to identify miRNAs characteristic for naive Treg population as well as for subpopulations of memory cells (Smigielska-Czepiel et al., 2014). Interestingly, miR-146a belongs to signature common to naïve and memory Treg, whereas miR-21 belongs to signature of T memory compartment (Smigielska-Czepiel et al., 2014). Of note, the miRNA repertoire of Tregs is reflected in their EVs (Torri et al., 2017).

Another interesting implication of circulating inflammamiR analysis can be envisaged in the evaluation of anti-inflammatory therapies efficacy. When serum miR-21-5p and miR-146a levels were analysed in twins discordant for estrogen-based hormone replacement therapy (HRT), their concentrations were lower in HRT users compared to their non-HRT using co-twins (Kangas et al., 2014).

Another example of the use of inflammamiRs as biomarkers of therapeutic intervention efficacy is suggested in our work concerning innovative insights into the molecular mechanisms by which the antioxidant molecule ubiquinol stems endothelial cell inflammatory responses and delays the SASP acquisition (Olivieri et al., 2013b). We also observed a correlation between miR-146a circulating levels and clinical efficacy of TNF-α inhibitor treatment in patients with psoriasis (Mensà et al., 2018). Quite similar results were reported in rheumatoid arthritis patients treated with anti-TNFα (Castro-Villegas et al., 2015) and in patients affected by myelodysplastic syndrome (Cerisoli et al., 2021). Furthermore, in our recent report previously cited on COVID-19, we observed that miR-146a levels in patients observed a significant increase of miR-146a serum levels only in patients classified as responders to anti-IL-6 receptor Tocilizumab treatment (Sabbatinelli et al., 2021). These data strongly support the hypothesis that the adverse outcomes of the current pandemic of COVID-19 could be related to the degree of inflammaging (Bonafe et al., 2020; de Candia et al., 2021).

Finally, few data were already reported on the association between genetic polymorphisms in gene coding for inflammamiRNAs, including miR-21-5p and miR-146a, and the chance to undergo successful aging (Franceschi et al., 2020). A common G/C SNP (rs2910164) of pri-mir-146a was first identified in samples of papillary thyroid carcinoma patients and the C allele was associated with a reduction of miRNA expression in human cells (Jazdzewski et al., 2008; Le et al., 2020; Papathanasiou et al., 2020). Not surprisingly, this SNP was associated with an increased risk to develop different types of cancers and coronary artery disease (CAD) (Bao et al., 2015; Lian et al., 2012; Wei et al., 2013; Xu et al., 2008). Notably, no conclusive results were

obtained regarding pri-miR146a SNP and risk of diabetes (Chen et al., 2019; Gholami et al., 2020). Interestingly, this pri-mir-146a SNP was recently associated with an overactive immune response and an increased risk to acquire infections (Keewan and Naser, 2020). This result is even more relevant in the current efforts to understand susceptibility to SARS-CoV-2 infection and the development of COVID-19 adverse outcomes.

Since the number of manuscripts reporting associations of circulating miR-21-5p and miR-146a levels with human disease is rapidly increasing, we reviewed the PubMed literature from 2010 to 2020 and listed the main studies on miR-146a and miR-21-5p circulating levels in patients affected by the most common ARDs, including T2DM, CVD and neurodegenerative diseases. Studies related to cancer were not included. We searched for 'human circulating miR-146a', 'human circulating miR-21', and 'T2DM' or 'CVD' or 'dementia' and selected the human-based research articles. Details on miR-146 studies are provided in **Table 1,** whereas those on miR-21 are reported in **Table 2.**

MiR-146a measured in serum/plasma showed a trend toward decreased levels in patients with T2DM compared to healthy controls, as also summarized in a meta-analysis (Alipoor et al., 2017), albeit miR-146a circulating levels are strongly related to patient age, gender, health status and disease complications. However, different relationships can be observed when considering whole blood (Morais Junior et al., 2017) or a peculiar population of EVs (Prattichizzo et al., 2021a), reinforcing the notion that different results are obtained when harvesting diverse blood components. Similarly, increased levels of miR-146a expression in CVD patients compared to healthy controls were reported (**Table 1**). Such data are particularly significant as CVDs represent a heterogeneous group of disorders; nevertheless, miR-146a appears a 'stable' indicator of the inflammatory status. Declining miR-146a trend from healthy condition to CVDs were reported only by two studies, one on hypertension (Hijmans et al., 2018) and the other on acute ischemic stroke (Li et al., 2017) in relatively small populations. Concerning dementia, studies on miR-146a levels in both plasma/serum and CSF analysed in patients with Alzheimer's disease reported non conclusive results. A possible explanation for these non-concordant results could be the different inclusion/exclusion criteria for the patient enrolment, as well as the small sized cohorts. Moreover, CSF withdrawal is a critical methodology and contamination of CSF with blood can influence study outcomes (Lusardi et al., 2017). Overall, even though the reproducibility of miRNA studies could be still a challenge, miR-146a seems to mark the age-dependent deviation from healthy trajectory.

Table 2 summarizes studies assessing miR-21-5p level in biological fluids, including serum, plasma, EVs, and cerebrospinal fluid (CSF) in T2DM, CVDs and neurodegenerative diseases. MiR-21-5p is currently one of the most broadly studied miRNAs in relation to CVD. Most of the studies – 19 out of 32 – showed an increase of the circulating miRNA level in patients compared to healthy controls. Only four studies reported a down-regulation of miR-21-5p, and one paper did not show a significant modulation (**Table 2**). The remaining seven studies showed a modulation of miR-21-5p between clinical subgroups of a specific disease. On the other hand, studies investigating miR-21-5p in T2DM and neurodegenerative diseases showed a greater level of heterogeneity. The variability in the results could be related to analytical issues, to the different sample source and to differences in the cohort composition in terms of specific disease features, e.g. disease duration, severity and time after diagnosis. Moreover, the relatively small sample sizes analysed in part of the studies makes difficult to draw definitive conclusions.

6. miR-21- and miR-146a-based therapeutic interventions

The extensive characterization of miR-146a function as a regulator of innate and adaptive immunity paved the way to multiple studies investigating its therapeutic potential in inflammatory dyfunctions, including ARDs. Event though miR-146a is induced as a compensatory response to acute inflammation, the extent of this response is often insufficient to restrain inflammation and to prevent the progression of organ damage (Bobba et al., 2021). In regard this issue, approaches that boost the endogenous miR-146a expression, or the selective delivery of miR-146a mimics, via either EVs or other nanoparticles, have been proposed and tested as below described.

Systemic administration of miR-146a has shown promising result in a murine model of diabetic peripheral neuropathy, a condition associated with marked neurovascular dysfunction. The suppression of IRAK1, TRAF6, and the downstream NF-kB pathway ameliorated endothelial dysfunction and improved peripheral nerve perfusion, independently of blood glucose control (Liu et al., 2017). Subsequent studies showed that, when administered in a form capable of crossing the blood-brain barrier, miR-146a could improve neurological functional outcomes also by mediating remyelination and oligodendrocyte differentiation, (Zhang et al., 2019). Accordingly, miR-146a administration has been demonstrated to prevent the detrimental outcomes related to the hyperinflammatory state associated with sepsis by promoting M2 polarization of splenic macrophages

(Funahashi et al., 2019). Interestingly, a similar effect was achieved when mesenchymal stem cells were stimulated by IL-1β, which results in the enhanced release of miR-146a-enriched EVs (Song et al., 2017). This evidence confirms that the intracellular mechanisms leading to miR-146a synthesis and release can be also exploited to obtain an increase of the endogenous levels of this miRNA. The relevance of restricting the delivery of miR-146a to specific cellular types comes from the conflicting results observed in osteoarthritis, a typical ARD. Indeed, intrarticular administration of miR-146a resulted in accelerated destruction of articular cartilage due to the suppression of major anabolic genes in chondrocytes (Zhang et al., 2017b). On the opposite, miR-146a delivery to $Ly6C^{high}$ monocytes, i.e. the murine counterpart of human classical monocytes, reduced osteoclast differentiation and bone erosion without affecting joint inflammation (Ammari et al., 2018).

The intranasal delivery of miR-146a agomir was able to improve behavioral and cognitive dysfunction and relieve the pathophysiological process in the AD mice model, suggesting the potential role of miR-146a as a new target for AD (Mai et al., 2019). Furthermore, miR-146a administration through AAV9 (adeno-associated virus serotype 9)-mediated gene delivery suggested potential therapeutic intervention in heart failure (HF), since an abnormal SUMOylation has emerged as a characteristic of HF pathology and miR-146a was identified as a SUMO1-targeting microRNA (Oh et al., 2018). As reported above, the delivery of DNA-oligonucleotide conjugated/miR-146a molecule to a scavenger receptor/Toll-like receptor 9 agonist was proved to be effective in animal and human models of NF-κB–driven inflammation and in myeloid leukemia (Su et al., 2020).

Promising results were obtained inhibiting miR-21 expression in diabetic wounds, liver steatosis (Afonso et al., 2018) and heart failure (Thum et al., 2008). Notably, encouraging results were also achieved by inducing, rather than inhibiting, miR-21 function. Indeed, the administration of engineered EVs containing miR-21 accelerated diabetic wound healing by promoting re-epithelialization and angiogenesis, with limited side effects and optimal miRNA bioavailability and intracellular delivery (Lv et al., 2020).

Despite broad clinical potential, only a few miRNA-based therapeutic agents entered clinical trials. Overall, the translation of miRNA-based therapeutics into the clinical practice is still hampered by many factors, including the rapid degradation of unmodified miRNA mimics/antagonists, the presence of biological barriers, and the saturation of the miRNA processing apparatus in specific cell types, which adds to the intrinsic immunogenicity of nucleic acids (Chen et al., 2015).

7. Conclusion and future perspectives

MiR-21 and miR-146 were first identified as immune system regulators in a systematic effort to find miRNAs able to influence the mammalian response to pro-inflammatory stimuli. These miRNAs can modulate inflammaging and were therefore named as inflammamiRs. Ten years of research efforts have substantiated the potential clinical relevance of inflammamiRs to represent both functional innovative biomarkers and therapeutic targets of inflammaging and ARDs.

A number of reasons have hampered until now the translation of circulating miR-21-5p and miR-146a as biomarkers for ARDs. The common view about the search of biomarkers usually advocates disease specificity. However, the gerontologic approach for ARDs prevention relies on the exactly opposite concept, i.e. a limited number of "pillars" of aging promote or predispose to the development of all the most common ARDs (Franceschi et al., 2018a; Furman et al., 2019). Thus, a biomarker that senses a pervasive phenomenon underlying the development of multiple diseases should not be used to predict specific ARD, rather to track deviations from a healthy aging trajectory (Olivieri et al., 2017). In addition, technical issues, mainly related to the normalization method and the standardization of procedures, characterized the field of circulating miRNAs as potential biomarkers (Micolucci et al., 2017; Prattichizzo et al., 2021b). Overall, some inflammamiRs, especially miR-146a and miR-21, are able to track physiological and pathological aging trajectories, and we believe that it will be possible in the next future to transfer these biomarkers from research to clinical diagnostics. Emerging knowledge on inflammamiRs might also support the researchers towards the exploration of how these findings can contribute to promote healthy aging and prevent or postpone ARD development.

Since aging occurs heterogeneously among subjects and across multiple cells and tissues in the same organism (Tuttle et al., 2020), it cannot be measured with a single unidimensional metric (Cohen et al., 2020; Schultz et al., 2020). The observation that multiple un-healthy conditions coexist in the same patients, namely multimorbidity (Johnston et al., 2019), supported the hypothesis of a continuum between healthy and unhealthy aging and suggested that the level of inflammaging – strictly related to biological age rather than chronological age – should be the best predictor of ARD development (Franceschi et al., 2018b). Accordingly, the clinical approach to the elderly patient needs to take into account that every patient is a world apart, and therefore the axiom of "one size fits all" is not applicable (Riondino et al., 2019). This tenet supports the increasing efforts

to develop precision medicine for elderly patients exploiting epigenetic markers such as miRNAs (Deelen et al., 2019; Lehallier et al., 2019; Tanaka et al., 2020).

Funding

This work was supported by the Italian Ministry of Health – Ricerca Corrente to IRCCS INRCA and IRCCS MultiMedica and by Università Politecnica delle Marche (RSA grant to FO).

Declarations of interest: none.

REFERENCES

Afonso, M.B., Rodrigues, P.M., Simao, A.L., Gaspar, M.M., Carvalho, T., Borralho, P., Banales, J.M., Castro, R.E., Rodrigues, C.M.P., 2018. miRNA-21 ablation protects against liver injury and necroptosis in cholestasis. Cell Death Differ 25, 857-872.

Akbaraly, T.N., Hamer, M., Ferrie, J.E., Lowe, G., Batty, G.D., Hagger-Johnson, G., Singh-Manoux, A., Shipley, M.J., Kivimaki, M., 2013. Chronic inflammation as a determinant of future aging phenotypes. CMAJ 185, E763-770.

Akira, S., Isshiki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T., Hirano, T., Kishimoto, T., 1990. A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family. EMBO J 9, 1897-1906.

Al-Hayali, M.A., Sozer, V., Durmus, S., Erdenen, F., Altunoglu, E., Gelisgen, R., Atukeren, P., Atak, P.G., Uzun, H., 2019. Clinical Value of Circulating Microribonucleic Acids miR-1 and miR-21 in Evaluating the Diagnosis of Acute Heart Failure in Asymptomatic Type 2 Diabetic Patients. Biomolecules 9.

Albani, D., Batelli, S., Polito, L., Prato, F., Pesaresi, M., Gajo, G.B., De Angeli, S., Zanardo, A., Galimberti, D., Scarpini, E., Gallucci, M., Forloni, G., 2009. Interleukin-6 plasma level increases with age in an Italian elderly population ("The Treviso Longeva"-Trelong-study) with a sex-specific contribution of rs1800795 polymorphism. Age (Dordr) 31, 155-162.

Alipoor, B., Ghaedi, H., Meshkani, R., Omrani, M.D., Sharifi, Z., Golmohammadi, T., 2018. The rs2910164 variant is associated with reduced miR-146a expression but not cytokine levels in patients with type 2 diabetes. J Endocrinol Invest 41, 557-566.

Alipoor, B., Ghaedi, H., Meshkani, R., Torkamandi, S., Saffari, S., Iranpour, M., Omrani, M.D., 2017. Association of MiR-146a Expression and Type 2 Diabetes Mellitus: A Meta-Analysis. Int J Mol Cell Med 6, 156-163.

Ammari, M., Presumey, J., Ponsolles, C., Roussignol, G., Roubert, C., Escriou, V., Toupet, K., Mausset-Bonnefont, A.L., Cren, M., Robin, M., Georgel, P., Nehmar, R., Taams, L., Grun, J., Grutzkau, A., Haupl, T., Pers, Y.M., Jorgensen, C., Duroux-Richard, I., Courties, G., Apparailly, F., 2018. Delivery of miR-146a to Ly6C(high) Monocytes Inhibits Pathogenic Bone Erosion in Inflammatory Arthritis. Theranostics 8, 5972-5985.

Androsavich, J.R., Chau, B.N., Bhat, B., Linsley, P.S., Walter, N.G., 2012. Disease-linked microRNA-21 exhibits drastically reduced mRNA binding and silencing activity in healthy mouse liver. RNA 18, 1510-1526.

Arroyo, A.B., Fernandez-Perez, M.P., Del Monte, A., Aguila, S., Mendez, R., Hernandez-Antolin, R., Garcia-Barber, N., de Los Reyes-Garcia, A.M., Gonzalez-Jimenez, P., Arcas, M.I., Vicente, V., Menendez, R., Andres, V., Gonzalez-Conejero, R., Martinez, C., 2020. miR-146a is a pivotal regulator of neutrophil extracellular trap formation promoting thrombosis. Haematologica.

Bagavad Gita, J., George, A.V., Pavithra, N., Chandrasekaran, S.C., Latchumanadhas, K., Gnanamani, A., 2019. Dysregulation of miR-146a by periodontal pathogens: A risk for acute coronary syndrome. J Periodontol 90, 756-765.

Balzano, F., Deiana, M., Dei Giudici, S., Oggiano, A., Pasella, S., Pinna, S., Mannu, A., Deiana, N., Porcu, B., Masala, A.G.E., Pileri, P.V., Scognamillo, F., Pala, C., Zinellu, A., Carru, C., Deiana, L., 2017. MicroRNA Expression Analysis of Centenarians and Rheumatoid Arthritis Patients Reveals a Common Expression Pattern. Int J Med Sci 14, 622-628.

Bao, M.H., Xiao, Y., Zhang, Q.S., Luo, H.Q., Luo, J., Zhao, J., Li, G.Y., Zeng, J., Li, J.M., 2015. Meta-Analysis of miR-146a Polymorphisms Association with Coronary Artery Diseases and Ischemic Stroke. Int J Mol Sci 16, 14305-14317.

Barwari, T., Eminaga, S., Mayr, U., Lu, R., Armstrong, P.C., Chan, M.V., Sahraei, M., Fernandez-Fuertes, M., Moreau, T., Barallobre-Barreiro, J., Lynch, M., Yin, X., Schulte, C., Baig, F., Pechlaner, R., Langley, S.R., Zampetaki, A., Santer, P., Weger, M., Plasenzotti, R., Schosserer, M., Grillari, J., Kiechl, S., Willeit, J., Shah, A.M., Ghevaert, C., Warner, T.D., Fernandez-Hernando, C., Suarez, Y., Mayr, M., 2018. Inhibition of profibrotic microRNA-21 affects platelets and their releasate. JCI Insight 3.

Beg, F., Wang, R., Saeed, Z., Devaraj, S., Masoor, K., Nakshatri, H., 2017. Inflammation-associated microRNA changes in circulating exosomes of heart failure patients. BMC Res Notes 10, 751.

Beinke, S., Ley, S.C., 2004. Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. Biochem J 382, 393-409.

Bejerano, T., Etzion, S., Elyagon, S., Etzion, Y., Cohen, S., 2018. Nanoparticle Delivery of miRNA-21 Mimic to Cardiac Macrophages Improves Myocardial Remodeling after Myocardial Infarction. Nano Lett 18, 5885-5891.

Benz, F., Roy, S., Trautwein, C., Roderburg, C., Luedde, T., 2016. Circulating MicroRNAs as Biomarkers for Sepsis. Int J Mol Sci 17.

Bernal, G.M., Wahlstrom, J.S., Crawley, C.D., Cahill, K.E., Pytel, P., Liang, H., Kang, S., Weichselbaum, R.R., Yamini, B., 2014. Loss of Nfkb1 leads to early onset aging. Aging (Albany NY) 6, 931-943.

Bertolet, G., Kongchan, N., Miller, R., Patel, R.K., Jain, A., Choi, J.M., Saltzman, A.B., Christenson, A., Jung, S.Y., Malovannaya, A., Grimson, A., Neilson, J.R., 2019. MiR-146a wild-type 3' sequence identity is dispensable for proper innate immune function in vivo. Life Sci Alliance 2.

Bhatt, K., Lanting, L.L., Jia, Y., Yadav, S., Reddy, M.A., Magilnick, N., Boldin, M., Natarajan, R., 2016. Anti-Inflammatory Role of MicroRNA-146a in the Pathogenesis of Diabetic Nephropathy. J Am Soc Nephrol 27, 2277-2288.

Bobba, C.M., Fei, Q., Shukla, V., Lee, H., Patel, P., Putman, R.K., Spitzer, C., Tsai, M., Wewers, M.D., Lee, R.J., Christman, J.W., Ballinger, M.N., Ghadiali, S.N., Englert, J.A., 2021. Nanoparticle delivery of microRNA-146a regulates mechanotransduction in lung macrophages and mitigates injury during mechanical ventilation. Nat Commun 12, 289.

Boele, J., Persson, H., Shin, J.W., Ishizu, Y., Newie, I.S., Sokilde, R., Hawkins, S.M., Coarfa, C., Ikeda, K., Takayama, K., Horie-Inoue, K., Ando, Y., Burroughs, A.M., Sasaki, C., Suzuki, C., Sakai, M., Aoki, S., Ogawa, A., Hasegawa, A., Lizio, M., Kaida, K., Teusink, B., Carninci, P., Suzuki, H., Inoue, S., Gunaratne, P.H., Rovira, C., Hayashizaki, Y., de Hoon, M.J., 2014. PAPD5-mediated 3' adenylation and subsequent degradation of miR-21 is disrupted in proliferative disease. Proc Natl Acad Sci U S A 111, 11467-11472.

Boldin, M.P., Taganov, K.D., Rao, D.S., Yang, L., Zhao, J.L., Kalwani, M., Garcia-Flores, Y., Luong, M., Devrekanli, A., Xu, J., Sun, G., Tay, J., Linsley, P.S., Baltimore, D., 2011. miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. J Exp Med 208, 1189-1201.

Bonafè, M., Olivieri, F., Cavallone, L., Giovagnetti, S., Marchegiani, F., Cardelli, M., Pieri, C., Marra, M., Antonicelli, R., Lisa, R., Rizzo, M.R., Paolisso, G., Monti, D., Franceschi, C., 2001. A gender-dependent genetic predisposition to produce high levels of IL-6 is detrimental for longevity. European Journal of Immunology 31, 2357-2361.

Bonafe, M., Olivieri, F., Cavallone, L., Giovagnetti, S., Mayegiani, F., Cardelli, M., Pieri, C., Marra, M., Antonicelli, R., Lisa, R., Rizzo, M.R., Paolisso, G., Monti, D., Franceschi, C., 2001. A gender--dependent genetic predisposition to produce high levels of IL-6 is detrimental for longevity. Eur J Immunol 31, 2357-2361.

Bonafe, M., Prattichizzo, F., Giuliani, A., Storci, G., Sabbatinelli, J., Olivieri, F., 2020. Inflamm-aging: Why older men are the most susceptible to SARS-CoV-2 complicated outcomes. Cytokine Growth Factor Rev 53, 33-37.

Burgos, K., Malenica, I., Metpally, R., Courtright, A., Rakela, B., Beach, T., Shill, H., Adler, C., Sabbagh, M., Villa, S., Tembe, W., Craig, D., Van Keuren-Jensen, K., 2014. Profiles of extracellular miRNA in cerebrospinal fluid and serum from patients with Alzheimer's and Parkinson's diseases correlate with disease status and features of pathology. PLoS One 9, e94839.

Burkitt, M.D., Williams, J.M., Townsend, T., Hough, R., Duckworth, C.A., Pritchard, D.M., 2017. Mice lacking NF-kappaB1 exhibit marked DNA damage responses and more severe gastric pathology in response to intraperitoneal tamoxifen administration. Cell Death Dis 8, e2939.

Burton, D.G.A., Stolzing, A., 2018. Cellular senescence: Immunosurveillance and future immunotherapy. Ageing Res Rev 43, 17-25.

Cakmak, H.A., Coskunpinar, E., Ikitimur, B., Barman, H.A., Karadag, B., Tiryakioglu, N.O., Kahraman, K., Vural, V.A., 2015. The prognostic value of circulating microRNAs in heart failure: preliminary results from a genome-wide expression study. J Cardiovasc Med (Hagerstown) 16, 431-437.

Canadas-Lozano, D., Marin-Aguilar, F., Castejon-Vega, B., Ryffel, B., Navarro-Pando, J.M., Ruiz-Cabello, J., Alcocer-Gomez, E., Bullon, P., Cordero, M.D., 2020. Blockade of the NLRP3 inflammasome improves metabolic health and lifespan in obese mice. Geroscience 42, 715-725.

Canfran-Duque, A., Rotllan, N., Zhang, X., Fernandez-Fuertes, M., Ramirez-Hidalgo, C., Araldi, E., Daimiel, L., Busto, R., Fernandez-Hernando, C., Suarez, Y., 2017. Macrophage deficiency of miR-21 promotes apoptosis, plaque necrosis, and vascular inflammation during atherogenesis. EMBO Mol Med 9, 1244-1262.

Capri, M., Santoro, A., Garagnani, P., Bacalini, M.G., Pirazzini, C., Olivieri, F., Procopio, A., Salvioli, S., Franceschi, C., 2014. Genes of human longevity: an endless quest? Curr Vasc Pharmacol 12, 707-717.

Carthew, R.W., Sontheimer, E.J., 2009. Origins and Mechanisms of miRNAs and siRNAs. Cell 136, 642-655.

Cartwright, T., Perkins, N.D., C, L.W., 2016. NFKB1: a suppressor of inflammation, ageing and cancer. FEBS J 283, 1812-1822.

Castro-Villegas, C., Perez-Sanchez, C., Escudero, A., Filipescu, I., Verdu, M., Ruiz-Limon, P., Aguirre, M.A., Jimenez-Gomez, Y., Font, P., Rodriguez-Ariza, A., Peinado, J.R., Collantes-Estevez, E., Gonzalez-Conejero, R., Martinez, C., Barbarroja, N., Lopez-Pedrera, C., 2015. Circulating miRNAs as potential biomarkers of therapy effectiveness in rheumatoid arthritis patients treated with anti-TNFalpha. Arthritis Res Ther 17, 49.

Cengiz, M., Yavuzer, S., Kilickiran Avci, B., Yuruyen, M., Yavuzer, H., Dikici, S.A., Karatas, O.F., Ozen, M., Uzun, H., Ongen, Z., 2015. Circulating miR-21 and eNOS in subclinical atherosclerosis in patients with hypertension. Clin Exp Hypertens 37, 643-649.

Cerisoli, S., Marinelli Busilacchi, E., Mattiucci, D., Rossi, E., Mariani, M., Guescini, M., Pugnaloni, A., Olivieri, F., Olivieri, A., Poloni, A., 2021. The exosomal surface phenotype and inflamma-miR cargo correlate with MDS diagnosis. Br J Haematol 192, e4-e7.

Chen, L., Yang, J., Lu, J., Cao, S., Zhao, Q., Yu, Z., 2018. Identification of aberrant circulating miRNAs in Parkinson's disease plasma samples. Brain Behav 8, e00941.

Chen, X., Wang, W., Li, R., Yu, J., Gao, L., 2019. Association between polymorphisms in microRNAs and susceptibility to diabetes mellitus: A meta-analysis. Medicine (Baltimore) 98, e17519.

Chen, Y., Gao, D.Y., Huang, L., 2015. In vivo delivery of miRNAs for cancer therapy: challenges and strategies. Adv Drug Deliv Rev 81, 128-141.
Chien, H.Y., Chen, C.Y., Chiu, Y.H., Lin, Y.C., Li, W.C., 2016. Differential microRNA Profiles Predict Diabetic Nephropathy Progression in Taiwan. Int J Med Sci 13, 457-465.
Chung, H.Y., Kim, D.H., Lee, E.K., Chung, K.W., Chung, S., Lee, B., Seo, A.Y., Chung, J.H., Jung, Y.S., Im, E., Lee, J., Kim, N.D., Choi, Y.J., Im, D.S., Yu, B.P., 2019. Redefining Chronic Inflammation in Aging and Age-Related Diseases: Proposal of the Senoinflammation Concept. Aging Dis 10, 367-382.
Coffey, S., Williams, M.J., Phillips, L.V., Jones, G.T., 2015. Circulating microRNA Profiling Needs Further Refinement Before Clinical Use in Patients With Aortic Stenosis. J Am Heart Assoc 4, e002150.
Cohen, A.A., Kennedy, B.K., Anglas, U., Bronikowski, A.M., Deelen, J., Dufour, F., Ferbeyre, G., Ferrucci, L., Franceschi, C., Frasca, D., Friguet, B., Gaudreau, P., Gladyshev, V.N., Gonos, E.S., Gorbunova, V., Gut, P., Ivanchenko, M., Legault, V., Lemaitre, J.F., Liontis, T., Liu, G.H., Liu, M., Maier, A.B., Nobrega, O.T., Olde Rikkert, M.G.M., Pawelec, G., Rheault, S., Senior, A.M., Simm, A., Soo, S., Traa, A., Ukraintseva, S., Vanhaelen, Q., Van Raamsdonk, J.M., Witkowski, J.M., Yashin, A.I., Ziman, R., Fulop, T., 2020. Lack of consensus on an aging biology paradigm? A global survey reveals an agreement to disagree, and the need for an interdisciplinary framework. Mech Ageing Dev 191, 111316.
Coppe, J.P., Patil, C.K., Rodier, F., Sun, Y., Munoz, D.P., Goldstein, J., Nelson, P.S., Desprez, P.Y., Campisi, J., 2008. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 6, 2853-2868.
d'Adhemar, C.J., Spillane, C.D., Gallagher, M.F., Bates, M., Costello, K.M., Barry-O'Crowley, J., Haley, K., Kernan, N., Murphy, C., Smyth, P.C., O'Byrne, K., Pennington, S., Cooke, A.A., Ffrench, B., Martin, C.M., O'Donnell, D., Hennessy, B., Stordal, B., Finn, S., McCann, A., Gleeson, N., D'Arcy, T., Flood, B., O'Neill, L.A., Sheils, O., O'Toole, S., O'Leary, J.J., 2014. The MyD88+ phenotype is an adverse prognostic factor in epithelial ovarian cancer. PLoS One 9, e100816.
da Silva, A.M.G., de Araujo, J.N.G., de Oliveira, K.M., Novaes, A.E.M., Lopes, M.B., de Sousa, J.C.V., Filho, A.A.A., Luchessi, A.D., de Rezende, A.A., Hirata, M.H., Silbiger, V.N., 2018. Circulating miRNAs in acute new-onset atrial fibrillation and their target mRNA network. J Cardiovasc Electrophysiol 29, 1159-1166.
Darabi, F., Aghaei, M., Movahedian, A., Pourmoghadas, A., Sarrafzadegan, N., 2016. The role of serum levels of microRNA-21 and matrix metalloproteinase-9 in patients with acute coronary syndrome. Mol Cell Biochem 422, 51-60.
Dayan, N., Schlosser, K., Stewart, D.J., Delles, C., Kaur, A., Pilote, L., 2018. Circulating MicroRNAs Implicate Multiple Atherogenic Abnormalities in the Long-Term Cardiovascular Sequelae of Preeclampsia. Am J Hypertens 31, 1093-1097.
de Candia, P., Prattichizzo, F., Garavelli, S., Matarese, G., 2021. T Cells: Warriors of SARS-CoV-2 Infection. Trends Immunol 42, 18-30.
De Martinis, M., Franceschi, C., Monti, D., Ginaldi, L., 2005. Inflamm-ageing and lifelong antigenic load as major determinants of ageing rate and longevity. FEBS Lett 579, 2035-2039.
Deelen, J., Kettunen, J., Fischer, K., van der Spek, A., Trompet, S., Kastenmuller, G., Boyd, A., Zierer, J., van den Akker, E.B., Ala-Korpela, M., Amin, N., Demirkan, A., Ghanbari, M., van Heemst, D., Ikram, M.A., van Klinken, J.B., Mooijaart, S.P., Peters, A., Salomaa, V., Sattar, N., Spector, T.D., Tiemeier, H., Verhoeven, A., Waldenberger, M., Wurtz, P., Davey Smith, G., Metspalu, A., Perola, M., Menni, C., Geleijnse, J.M., Drenos, F., Beekman, M., Jukema, J.W., van Duijn, C.M., Slagboom, P.E., 2019. A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals. Nat Commun 10, 3346.
Denk, J., Boelmans, K., Siegismund, C., Lassner, D., Arlt, S., Jahn, H., 2015. MicroRNA Profiling of CSF Reveals Potential Biomarkers to Detect Alzheimer`s Disease. PLoS One 10, e0126423.
DiDonato, J.A., Hayakawa, M., Rothwarf, D.M., Zandi, E., Karin, M., 1997. A cytokine-responsive IkappaB kinase that activates the transcription factor NF-kappaB. Nature 388, 548-554.
Ding, H., Wang, Y., Hu, L., Xue, S., Wang, Y., Zhang, L., Zhang, Y., Qi, H., Yu, H., Aung, L.H.H., An, Y., Li, P., 2020a. Combined detection of miR-21-5p, miR-30a-3p, miR-30a-5p, miR-155-5p, miR-216a and miR-217 for screening of early heart failure diseases. Biosci Rep 40.
Ding, X., Jing, N., Shen, A., Guo, F., Song, Y., Pan, M., Ma, X., Zhao, L., Zhang, H., Wu, L., Qin, G., Zhao, Y., 2020b. MiR-21-5p in macrophage-derived extracellular vesicles affects podocyte pyroptosis in diabetic nephropathy by regulating A20. J Endocrinol Invest.
Dong, H., Li, J., Huang, L., Chen, X., Li, D., Wang, T., Hu, C., Xu, J., Zhang, C., Zen, K., Xiao, S., Yan, Q., Wang, C., Zhang, C.Y., 2015. Serum MicroRNA Profiles Serve as Novel Biomarkers for the Diagnosis of Alzheimer's Disease. Dis Markers 2015, 625659.
Dong, S., Cheng, Y., Yang, J., Li, J., Liu, X., Wang, X., Wang, D., Krall, T.J., Delphin, E.S., Zhang, C., 2009. MicroRNA expression signature and the role of microRNA-21 in the early phase of acute myocardial infarction. J Biol Chem 284, 29514-29525.
Dufey, E., Bravo-San Pedro, J.M., Eggers, C., Gonzalez-Quiroz, M., Urra, H., Sagredo, A.I., Sepulveda, D., Pihan, P., Carreras-Sureda, A., Hazari, Y., Sagredo, E.A., Gutierrez, D., Valls, C., Papaioannou, A., Acosta-Alvear, D., Campos, G., Domingos, P.M., Pedeux, R., Chevet, E., Alvarez, A., Godoy, P., Walter, P., Glavic, A., Kroemer,

G., Hetz, C., 2020. Genotoxic stress triggers the activation of IRE1alpha-dependent RNA decay to modulate the DNA damage response. Nat Commun 11, 2401.
Ershler, W.B., 1993. Interleukin-6: a cytokine for gerontologists. J Am Geriatr Soc 41, 176-181.
Ertekin, S., Yildirim, O., Dinc, E., Ayaz, L., Fidanci, S.B., Tamer, L., 2014. Evaluation of circulating miRNAs in wet age-related macular degeneration. Mol Vis 20, 1057-1066.
Etzrodt, M., Cortez-Retamozo, V., Newton, A., Zhao, J., Ng, A., Wildgruber, M., Romero, P., Wurdinger, T., Xavier, R., Geissmann, F., Meylan, E., Nahrendorf, M., Swirski, F.K., Baltimore, D., Weissleder, R., Pittet, M.J., 2012. Regulation of monocyte functional heterogeneity by miR-146a and Relb. Cell Rep 1, 317-324.
Fabbri, M., Paone, A., Calore, F., Galli, R., Gaudio, E., Santhanam, R., Lovat, F., Fadda, P., Mao, C., Nuovo, G.J., Zanesi, N., Crawford, M., Ozer, G.H., Wernicke, D., Alder, H., Caligiuri, M.A., Nana-Sinkam, P., Perrotti, D., Croce, C.M., 2012. MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. Proc Natl Acad Sci U S A 109, E2110-2116.
Fafian-Labora, J.A., O'Loghlen, A., 2020. Classical and Nonclassical Intercellular Communication in Senescence and Ageing. Trends Cell Biol 30, 628-639.
Fan, K.L., Zhang, H.F., Shen, J., Zhang, Q., Li, X.L., 2013. Circulating microRNAs levels in Chinese heart failure patients caused by dilated cardiomyopathy. Indian Heart J 65, 12-16.
Fang, L., Ellims, A.H., Moore, X.L., White, D.A., Taylor, A.J., Chin-Dusting, J., Dart, A.M., 2015. Circulating microRNAs as biomarkers for diffuse myocardial fibrosis in patients with hypertrophic cardiomyopathy. J Transl Med 13, 314.
Feng, Y., Chen, L., Luo, Q., Wu, M., Chen, Y., Shi, X., 2018. Involvement of microRNA-146a in diabetic peripheral neuropathy through the regulation of inflammation. Drug Des Devel Ther 12, 171-177.
Ferrucci, L., Corsi, A., Lauretani, F., Bandinelli, S., Bartali, B., Taub, D.D., Guralnik, J.M., Longo, D.L., 2005. The origins of age-related proinflammatory state. Blood 105, 2294-2299.
Ferrucci, L., Fabbri, E., 2018. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat Rev Cardiol 15, 505-522.
Fielder, E., Tweedy, C., Wilson, C., Oakley, F., LeBeau, F.E.N., Passos, J.F., Mann, D.A., von Zglinicki, T., Jurk, D., 2020. Anti-inflammatory treatment rescues memory deficits during aging in nfkb1(-/-) mice. Aging Cell 19, e13188.
Franceschi, C., Bonafe, M., 2003. Centenarians as a model for healthy aging. Biochem Soc Trans 31, 457-461.
Franceschi, C., Bonafe, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000a. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 908, 244-254.
Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000b. Inflamm-aging. An evolutionary perspective on immunosenescence, Annals of the New York Academy of Sciences, pp. 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. Mechanisms of Ageing and Development 128, 92-105.
Franceschi, C., Cossarizza, A., 1995. Introduction: the reshaping of the immune system with age. Int Rev Immunol 12, 1-4.
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.
Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., Santoro, A., 2018a. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. Nat Rev Endocrinol 14, 576-590.
Franceschi, C., Garagnani, P., Vitale, G., Capri, M., Salvioli, S., 2017. Inflammaging and 'Garb-aging'. Trends Endocrinol Metab 28, 199-212.
Franceschi, C., Zaikin, A., Gordleeva, S., Ivanchenko, M., Bonifazi, F., Storci, G., Bonafe, M., 2018b. Inflammaging 2018: An update and a model. Semin Immunol 40, 1-5.
Fu, Y., Zhen, J., Lu, Z., 2017. Synergetic Neuroprotective Effect of Docosahexaenoic Acid and Aspirin in SH-Y5Y by Inhibiting miR-21 and Activating RXRalpha and PPARalpha. DNA Cell Biol 36, 482-489.
Fujita, S., Ito, T., Mizutani, T., Minoguchi, S., Yamamichi, N., Sakurai, K., Iba, H., 2008. miR-21 Gene expression triggered by AP-1 is sustained through a double-negative feedback mechanism. J Mol Biol 378, 492-504.
Fulop, T., Larbi, A., Dupuis, G., Le Page, A., Frost, E.H., Cohen, A.A., Witkowski, J.M., Franceschi, C., 2018. Immunosenescence and Inflamm-Aging As Two Sides of the Same Coin: Friends or Foes? Frontiers in immunology 8, 1960-1960.
Funahashi, Y., Kato, N., Masuda, T., Nishio, F., Kitai, H., Ishimoto, T., Kosugi, T., Tsuboi, N., Matsuda, N., Maruyama, S., Kadomatsu, K., 2019. miR-146a targeted to splenic macrophages prevents sepsis-induced multiple organ injury. Lab Invest 99, 1130-1142.
Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D.W., Fasano, A., Miller, G.W., Miller, A.H., Mantovani, A., Weyand, C.M., Barzilai, N., Goronzy, J.J., Rando, T.A., Effros, R.B., Lucia, A., Kleinstreuer, N., Slavich, G.M., 2019. Chronic inflammation in the etiology of disease across the life span. Nat Med 25, 1822-1832.

Gamez-Valero, A., Campdelacreu, J., Vilas, D., Ispierto, L., Rene, R., Alvarez, R., Armengol, M.P., Borras, F.E., Beyer, K., 2019. Exploratory study on microRNA profiles from plasma-derived extracellular vesicles in Alzheimer's disease and dementia with Lewy bodies. Transl Neurodegener 8, 31.

Garbers, C., Hermanns, H.M., Schaper, F., Muller-Newen, G., Grotzinger, J., Rose-John, S., Scheller, J., 2012. Plasticity and cross-talk of interleukin 6-type cytokines. Cytokine Growth Factor Rev 23, 85-97.

Garcia-Jacobo, R.E., Uresti-Rivera, E.E., Portales-Perez, D.P., Gonzalez-Amaro, R., Lara-Ramirez, E.E., Enciso-Moreno, J.A., Garcia-Hernandez, M.H., 2019. Circulating miR-146a, miR-34a and miR-375 in type 2 diabetes patients, pre-diabetic and normal-glycaemic individuals in relation to beta-cell function, insulin resistance and metabolic parameters. Clin Exp Pharmacol Physiol 46, 1092-1100.

Gholami, M., Asgarbeik, S., Razi, F., Esfahani, E.N., Zoughi, M., Vahidi, A., Larijani, B., Amoli, M.M., 2020. Association of microRNA gene polymorphisms with Type 2 diabetes mellitus: A systematic review and meta-analysis. J Res Med Sci 25, 56.

Giovannini, S., Onder, G., Liperoti, R., Russo, A., Carter, C., Capoluongo, E., Pahor, M., Bernabei, R., Landi, F., 2011. Interleukin-6, C-reactive protein, and tumor necrosis factor-alpha as predictors of mortality in frail, community-living elderly individuals. J Am Geriatr Soc 59, 1679-1685.

Giuliani, A., Cirilli, I., Prattichizzo, F., Mensa, E., Fulgenzi, G., Sabbatinelli, J., Graciotti, L., Olivieri, F., Procopio, A.D., Tiano, L., Rippo, M.R., 2018. The mitomiR/Bcl-2 axis affects mitochondrial function and autophagic vacuole formation in senescent endothelial cells. Aging (Albany NY) 10, 2855-2873.

Giuliani, A., Gaetani, S., Sorgentoni, G., Agarbati, S., Laggetta, M., Matacchione, G., Gobbi, M., Rossi, T., Galeazzi, R., Piccinini, G., Pelliccioni, G., Bonfigli, A.R., Procopio, A.D., Albertini, M.C., Sabbatinelli, J., Olivieri, F., Fazioli, F., 2021. Circulating Inflamma-miRs as Potential Biomarkers of Cognitive Impairment in Patients Affected by Alzheimer's Disease. Frontiers in Aging Neuroscience 13.

Giuliani, A., Londin, E., Ferracin, M., Mensa, E., Prattichizzo, F., Ramini, D., Marcheselli, F., Recchioni, R., Rippo, M.R., Bonafe, M., Rigoutsos, I., Olivieri, F., Sabbatinelli, J., 2020. Long-term exposure of human endothelial cells to metformin modulates miRNAs and isomiRs. Sci Rep 10, 21782.

Gong, J., Sun, D., 2020. Study on the mechanism of curcumin to reduce the inflammatory response of temporal lobe in Alzheimer's disease by regulating miR-146a. Minerva Med.

Gonzalez-Quiroz, M., Blondel, A., Sagredo, A., Hetz, C., Chevet, E., Pedeux, R., 2020. When Endoplasmic Reticulum Proteostasis Meets the DNA Damage Response. Trends Cell Biol 30, 881-891.

Grabmaier, U., Clauss, S., Gross, L., Klier, I., Franz, W.M., Steinbeck, G., Wakili, R., Theiss, H.D., Brenner, C., 2017. Diagnostic and prognostic value of miR-1 and miR-29b on adverse ventricular remodeling after acute myocardial infarction - The SITAGRAMI-miR analysis. Int J Cardiol 244, 30-36.

Grants, J.M., Wegrzyn, J., Hui, T., O'Neill, K., Shadbolt, M., Knapp, D., Parker, J., Deng, Y., Gopal, A., Docking, T.R., Fuller, M., Li, J., Boldin, M., Eaves, C.J., Hirst, M., Karsan, A., 2020. Altered microRNA expression links IL6 and TNF-induced inflammaging with myeloid malignancy in humans and mice. Blood 135, 2235-2251.

Gritsenko, A., Green, J.P., Brough, D., Lopez-Castejon, G., 2020. Mechanisms of NLRP3 priming in inflammaging and age related diseases. Cytokine Growth Factor Rev 55, 15-25.

Gu, G.L., Xu, X.L., Sun, X.T., Zhang, J., Guo, C.F., Wang, C.S., Sun, B., Guo, G.L., Ma, K., Huang, Y.Y., Sun, L.Q., Wang, Y.Q., 2015. Cardioprotective Effect of MicroRNA-21 in Murine Myocardial Infarction. Cardiovasc Ther 33, 109-117.

Guan, Y.J., Li, J., Yang, X., Du, S., Ding, J., Gao, Y., Zhang, Y., Yang, K., Chen, Q., 2018. Evidence that miR-146a attenuates aging- and trauma-induced osteoarthritis by inhibiting Notch1, IL-6, and IL-1 mediated catabolism. Aging Cell 17, e12752.

Guerville, F., De Souto Barreto, P., Ader, I., Andrieu, S., Casteilla, L., Dray, C., Fazilleau, N., Guyonnet, S., Langin, D., Liblau, R., Parini, A., Valet, P., Vergnolle, N., Rolland, Y., Vellas, B., 2020. Revisiting the Hallmarks of Aging to Identify Markers of Biological Age. J Prev Alzheimers Dis 7, 56-64.

Hamczyk, M.R., Nevado, R.M., Barettino, A., Fuster, V., Andres, V., 2020. Biological Versus Chronological Aging: JACC Focus Seminar. J Am Coll Cardiol 75, 919-930.

Han, H., Qu, G., Han, C., Wang, Y., Sun, T., Li, F., Wang, J., Luo, S., 2015. MiR-34a, miR-21 and miR-23a as potential biomarkers for coronary artery disease: a pilot microarray study and confirmation in a 32 patient cohort. Exp Mol Med 47, e138.

Hattori, T., Ohoka, N., Hayashi, H., Onozaki, K., 2003. C/EBP homologous protein (CHOP) up-regulates IL-6 transcription by trapping negative regulating NF-IL6 isoform. FEBS Lett 541, 33-39.

Hayden, M.S., Ghosh, S., 2004. Signaling to NF-kappaB. Genes Dev 18, 2195-2224.

He, Y., Sun, X., Huang, C., Long, X.R., Lin, X., Zhang, L., Lv, X.W., Li, J., 2014. MiR-146a regulates IL-6 production in lipopolysaccharide-induced RAW264.7 macrophage cells by inhibiting Notch1. Inflammation 37, 71-82.

Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., Lipford, G., Wagner, H., Bauer, S., 2004. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 303, 1526-1529.

Hijmans, J.G., Diehl, K.J., Bammert, T.D., Kavlich, P.J., Lincenberg, G.M., Greiner, J.J., Stauffer, B.L., DeSouza, C.A., 2018. Association between hypertension and circulating vascular-related microRNAs. J Hum Hypertens 32, 440-447.

Hoffmann, A., Leung, T.H., Baltimore, D., 2003. Genetic analysis of NF-kappaB/Rel transcription factors defines functional specificities. EMBO J 22, 5530-5539.
Jaillon, S., Berthenet, K., Garlanda, C., 2019. Sexual Dimorphism in Innate Immunity. Clin Rev Allergy Immunol 56, 308-321.
Jazdzewski, K., Murray, E.L., Franssila, K., Jarzab, B., Schoenberg, D.R., de la Chapelle, A., 2008. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci U S A 105, 7269-7274.
Jenike, A.E., Halushka, M.K., 2021. miR-21: a non-specific biomarker of all maladies. Biomark Res 9, 18.
Jiang, M., Xiang, Y., Wang, D., Gao, J., Liu, D., Liu, Y., Liu, S., Zheng, D., 2012. Dysregulated expression of miR-146a contributes to age-related dysfunction of macrophages. Aging Cell 11, 29-40.
Jo, E.K., Kim, J.K., Shin, D.M., Sasakawa, C., 2016. Molecular mechanisms regulating NLRP3 inflammasome activation. Cell Mol Immunol 13, 148-159.
Johnston, M.C., Crilly, M., Black, C., Prescott, G.J., Mercer, S.W., 2019. Defining and measuring multimorbidity: a systematic review of systematic reviews. Eur J Public Health 29, 182-189.
Jurk, D., Wilson, C., Passos, J.F., Oakley, F., Correia-Melo, C., Greaves, L., Saretzki, G., Fox, C., Lawless, C., Anderson, R., Hewitt, G., Pender, S.L., Fullard, N., Nelson, G., Mann, J., van de Sluis, B., Mann, D.A., von Zglinicki, T., 2014. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. Nat Commun 2, 4172.
Kangas, R., Pöllänen, E., Rippo, M.R., Lanzarini, C., Prattichizzo, F., Niskala, P., Jylhävä, J., Sipilä, S., Kaprio, J., Procopio, A.D., Capri, M., Franceschi, C., Olivieri, F., Kovanen, V., 2014. Circulating miR-21, miR-146a and Fas ligand respond to postmenopausal estrogen-based hormone replacement therapy - A study with monozygotic twin pairs. Mechanisms of Ageing and Development 143-144, 1-8.
Kangas, R., Tormakangas, T., Heinonen, A., Alen, M., Suominen, H., Kovanen, V., Laakkonen, E.K., Korhonen, M.T., 2017. Declining Physical Performance Associates with Serum FasL, miR-21, and miR-146a in Aging Sprinters. Biomed Res Int 2017, 8468469.
Karin, M., Ben-Neriah, Y., 2000. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. Annu Rev Immunol 18, 621-663.
Keewan, E., Naser, S.A., 2020. MiR-146a rs2910164 G > C polymorphism modulates Notch-1/IL-6 signaling during infection: a possible risk factor for Crohn's disease. Gut Pathog 12, 48.
Kelley, N., Jeltema, D., Duan, Y., He, Y., 2019. The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. Int J Mol Sci 20.
Kelly, B., O'Neill, L.A., 2015. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Res 25, 771-784.
Kiko, T., Nakagawa, K., Tsuduki, T., Furukawa, K., Arai, H., Miyazawa, T., 2014. MicroRNAs in plasma and cerebrospinal fluid as potential markers for Alzheimer's disease. J Alzheimers Dis 39, 253-259.
Kim, O.Y., Chae, J.S., Paik, J.K., Seo, H.S., Jang, Y., Cavaillon, J.M., Lee, J.H., 2012. Effects of aging and menopause on serum interleukin-6 levels and peripheral blood mononuclear cell cytokine production in healthy nonobese women. Age (Dordr) 34, 415-425.
Kobayashi, H., Chang, S.H., Mori, D., Itoh, S., Hirata, M., Hosaka, Y., Taniguchi, Y., Okada, K., Mori, Y., Yano, F., Chung, U.I., Akiyama, H., Kawaguchi, H., Tanaka, S., Saito, T., 2016. Biphasic regulation of chondrocytes by Rela through induction of anti-apoptotic and catabolic target genes. Nat Commun 7, 13336.
Koppers-Lalic, D., Hackenberg, M., Bijnsdorp, I.V., van Eijndhoven, M.A.J., Sadek, P., Sie, D., Zini, N., Middeldorp, J.M., Ylstra, B., de Menezes, R.X., Wurdinger, T., Meijer, G.A., Pegtel, D.M., 2014. Nontemplated nucleotide additions distinguish the small RNA composition in cells from exosomes. Cell Rep 8, 1649-1658.
Kuilman, T., Michaloglou, C., Vredeveld, L.C., Douma, S., van Doorn, R., Desmet, C.J., Aarden, L.A., Mooi, W.J., Peeper, D.S., 2008. Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. Cell 133, 1019-1031.
Kura, B., Kalocayova, B., Devaux, Y., Bartekova, M., 2020. Potential Clinical Implications of miR-1 and miR-21 in Heart Disease and Cardioprotection. Int J Mol Sci 21.
Kurosawa, M., Arakaki, R., Yamada, A., Tsunematsu, T., Kudo, Y., Sprent, J., Ishimaru, N., 2017. NF-kappaB2 Controls the Migratory Activity of Memory T Cells by Regulating Expression of CXCR4 in a Mouse Model of Sjogren's Syndrome. Arthritis Rheumatol 69, 2193-2202.
La Sala, L., Mrakic-Sposta, S., Micheloni, S., Prattichizzo, F., Ceriello, A., 2018. Glucose-sensing microRNA-21 disrupts ROS homeostasis and impairs antioxidant responses in cellular glucose variability. Cardiovasc Diabetol 17, 105.
La Sala, L., Mrakic-Sposta, S., Tagliabue, E., Prattichizzo, F., Micheloni, S., Sangalli, E., Specchia, C., Uccellatore, A.C., Lupini, S., Spinetti, G., de Candia, P., Ceriello, A., 2019. Circulating microRNA-21 is an early predictor of ROS-mediated damage in subjects with high risk of developing diabetes and in drug-naive T2D. Cardiovasc Diabetol 18, 18.
Lara, P.C., Macias-Verde, D., Burgos-Burgos, J., 2020. Age-induced NLRP3 Inflammasome Over-activation Increases Lethality of SARS-CoV-2 Pneumonia in Elderly Patients. Aging Dis 11, 756-762.
Le, C.T., Nguyen, T.L., Nguyen, T.D., Nguyen, T.A., 2020. Human disease-associated single nucleotide polymorphism changes the orientation of DROSHA on pri-mir-146a. RNA 26, 1777-1786.

Lee, E.G., Boone, D.L., Chai, S., Libby, S.L., Chien, M., Lodolce, J.P., Ma, A., 2000. Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. Science 289, 2350-2354.
Lehallier, B., Gate, D., Schaum, N., Nanasi, T., Lee, S.E., Yousef, H., Moran Losada, P., Berdnik, D., Keller, A., Verghese, J., Sathyan, S., Franceschi, C., Milman, S., Barzilai, N., Wyss-Coray, T., 2019. Undulating changes in human plasma proteome profiles across the lifespan. Nat Med 25, 1843-1850.
Leng, S., Xue, Q.L., Huang, Y., Semba, R., Chaves, P., Bandeen-Roche, K., Fried, L., Walston, J., 2005. Total and differential white blood cell counts and their associations with circulating interleukin-6 levels in community-dwelling older women. J Gerontol A Biol Sci Med Sci 60, 195-199.
Li, K., Ching, D., Luk, F.S., Raffai, R.L., 2015a. Apolipoprotein E enhances microRNA-146a in monocytes and macrophages to suppress nuclear factor-kappaB-driven inflammation and atherosclerosis. Circ Res 117, e1-e11.
Li, S.H., Chen, L., Pang, X.M., Su, S.Y., Zhou, X., Chen, C.Y., Huang, L.G., Li, J.P., Liu, J.L., 2017. Decreased miR-146a expression in acute ischemic stroke directly targets the Fbxl10 mRNA and is involved in modulating apoptosis. Neurochem Int 107, 156-167.
Li, S.H., Su, S.Y., Liu, J.L., 2015b. Differential Regulation of microRNAs in Patients with Ischemic Stroke. Curr Neurovasc Res 12, 214-221.
Lian, H., Wang, L., Zhang, J., 2012. Increased risk of breast cancer associated with CC genotype of Has-miR-146a Rs2910164 polymorphism in Europeans. PLoS One 7, e31615.
Liang, Y., Zhou, Y., Shen, P., 2004. NF-kappaB and its regulation on the immune system. Cell Mol Immunol 1, 343-350.
Libermann, T.A., Baltimore, D., 1990. Activation of interleukin-6 gene expression through the NF-kappa B transcription factor. Mol Cell Biol 10, 2327-2334.
Lin, H., Joehanes, R., Pilling, L.C., Dupuis, J., Lunetta, K.L., Ying, S.X., Benjamin, E.J., Hernandez, D., Singleton, A., Melzer, D., Munson, P.J., Levy, D., Ferrucci, L., Murabito, J.M., 2014. Whole blood gene expression and interleukin-6 levels. Genomics 104, 490-495.
Liu, X., Dong, Y., Chen, S., Zhang, G., Zhang, M., Gong, Y., Li, X., 2015. Circulating MicroRNA-146a and MicroRNA-21 Predict Left Ventricular Remodeling after ST-Elevation Myocardial Infarction. Cardiology 132, 233-241.
Liu, X.S., Fan, B., Szalad, A., Jia, L., Wang, L., Wang, X., Pan, W., Zhang, L., Zhang, R., Hu, J., Zhang, X.M., Chopp, M., Zhang, Z.G., 2017. MicroRNA-146a Mimics Reduce the Peripheral Neuropathy in Type 2 Diabetic Mice. Diabetes 66, 3111-3121.
Loher, P., Londin, E.R., Rigoutsos, I., 2014. IsomiR expression profiles in human lymphoblastoid cell lines exhibit population and gender dependencies. Oncotarget 5, 8790-8802.
Low, J.T., Christie, M., Ernst, M., Dumoutier, L., Preaudet, A., Ni, Y., Griffin, M.D.W., Mielke, L.A., Strasser, A., Putoczki, T.L., O'Reilly, L.A., 2020. Loss of NFKB1 Results in Expression of Tumor Necrosis Factor and Activation of Signal Transducer and Activator of Transcription 1 to Promote Gastric Tumorigenesis in Mice. Gastroenterology 159, 1444-1458 e1415.
Luly, F.R., Leveque, M., Licursi, V., Cimino, G., Martin-Chouly, C., Theret, N., Negri, R., Cavinato, L., Ascenzioni, F., Del Porto, P., 2019. MiR-146a is over-expressed and controls IL-6 production in cystic fibrosis macrophages. Sci Rep 9, 16259.
Lusardi, T.A., Phillips, J.I., Wiedrick, J.T., Harrington, C.A., Lind, B., Lapidus, J.A., Quinn, J.F., Saugstad, J.A., 2017. MicroRNAs in Human Cerebrospinal Fluid as Biomarkers for Alzheimer's Disease. J Alzheimers Dis 55, 1223-1233.
Lv, Q., Deng, J., Chen, Y., Wang, Y., Liu, B., Liu, J., 2020. Engineered Human Adipose Stem-Cell-Derived Exosomes Loaded with miR-21-5p to Promote Diabetic Cutaneous Wound Healing. Mol Pharm 17, 1723-1733.
Ma, J., Liu, C., Yang, Y., Yu, J., Yang, J., Yu, S., Zhang, J., Huang, L., 2018. C/EBPbeta Acts Upstream of NF-kappaB P65 Subunit in Ox-LDL-Induced IL-1beta Production by Macrophages. Cell Physiol Biochem 48, 1605-1615.
Maggio, M., Guralnik, J.M., Longo, D.L., Ferrucci, L., 2006. Interleukin-6 in aging and chronic disease: a magnificent pathway. J Gerontol A Biol Sci Med Sci 61, 575-584.
Magilnick, N., Reyes, E.Y., Wang, W.L., Vonderfecht, S.L., Gohda, J., Inoue, J.I., Boldin, M.P., 2017. miR-146a-Traf6 regulatory axis controls autoimmunity and myelopoiesis, but is dispensable for hematopoietic stem cell homeostasis and tumor suppression. Proc Natl Acad Sci U S A 114, E7140-E7149.
Mai, H., Fan, W., Wang, Y., Cai, Y., Li, X., Chen, F., Chen, X., Yang, J., Tang, P., Chen, H., Zou, T., Hong, T., Wan, C., Zhao, B., Cui, L., 2019. Intranasal Administration of miR-146a Agomir Rescued the Pathological Process and Cognitive Impairment in an AD Mouse Model. Mol Ther Nucleic Acids 18, 681-695.
Man, S.M., Kanneganti, T.D., 2015. Regulation of inflammasome activation. Immunol Rev 265, 6-21.
Marin-Aguilar, F., Castejon-Vega, B., Alcocer-Gomez, E., Lendines-Cordero, D., Cooper, M.A., de la Cruz, P., Andujar-Pulido, E., Perez-Alegre, M., Muntane, J., Perez-Pulido, A.J., Ryffel, B., Robertson, A.A.B., Ruiz-Cabello, J., Bullon, P., Cordero, M.D., 2020a. NLRP3 Inflammasome Inhibition by MCC950 in Aged Mice Improves Health via Enhanced Autophagy and PPARalpha Activity. J Gerontol A Biol Sci Med Sci 75, 1457-1464.
Marin-Aguilar, F., Lechuga-Vieco, A.V., Alcocer-Gomez, E., Castejon-Vega, B., Lucas, J., Garrido, C., Peralta-Garcia, A., Perez-Pulido, A.J., Varela-Lopez, A., Quiles, J.L., Ryffel, B., Flores, I., Bullon, P., Ruiz-Cabello, J., Cordero, M.D., 2020b. NLRP3 inflammasome suppression improves longevity and prevents cardiac aging in male mice. Aging Cell 19, e13050.

Marquez, E.J., Chung, C.H., Marches, R., Rossi, R.J., Nehar-Belaid, D., Eroglu, A., Mellert, D.J., Kuchel, G.A., Banchereau, J., Ucar, D., 2020. Sexual-dimorphism in human immune system aging. Nat Commun 11, 751.

Matsuhashi, S., Manirujjaman, M., Hamajima, H., Ozaki, I., 2019. Control Mechanisms of the Tumor Suppressor PDCD4: Expression and Functions. Int J Mol Sci 20.

McManus, D.D., Tanriverdi, K., Lin, H., Esa, N., Kinno, M., Mandapati, D., Tam, S., Okike, O.N., Ellinor, P.T., Keaney, J.F., Jr., Donahue, J.K., Benjamin, E.J., Freedman, J.E., 2015. Plasma microRNAs are associated with atrial fibrillation and change after catheter ablation (the miRhythm study). Heart Rhythm 12, 3-10.

McMillan, D.H., Woeller, C.F., Thatcher, T.H., Spinelli, S.L., Maggirwar, S.B., Sime, P.J., Phipps, R.P., 2013. Attenuation of inflammatory mediator production by the NF-kappaB member RelB is mediated by microRNA-146a in lung fibroblasts. Am J Physiol Lung Cell Mol Physiol 304, L774-781.

Mensà, E., Giuliani, A., Matacchione, G., Gurău, F., Bonfigli, A.R., Romagnoli, F., De Luca, M., Sabbatinelli, J., Olivieri, F., 2019. Circulating miR-146a in healthy aging and type 2 diabetes: Age- and gender-specific trajectories. Mech Ageing Dev 180, 1-10.

Mensa, E., Guescini, M., Giuliani, A., Bacalini, M.G., Ramini, D., Corleone, G., Ferracin, M., Fulgenzi, G., Graciotti, L., Prattichizzo, F., Sorci, L., Battistelli, M., Monsurro, V., Bonfigli, A.R., Cardelli, M., Recchioni, R., Marcheselli, F., Latini, S., Maggio, S., Fanelli, M., Amatori, S., Storci, G., Ceriello, A., Stocchi, V., De Luca, M., Magnani, L., Rippo, M.R., Procopio, A.D., Sala, C., Budimir, I., Bassi, C., Negrini, M., Garagnani, P., Franceschi, C., Sabbatinelli, J., Bonafe, M., Olivieri, F., 2020. Small extracellular vesicles deliver miR-21 and miR-217 as pro-senescence effectors to endothelial cells. J Extracell Vesicles 9, 1725285.

Mensà, E., Recchioni, R., Marcheselli, F., Giuliodori, K., Consales, V., Molinelli, E., Prattichizzo, F., Rippo, M.R., Campanati, A., Procopio, A.D., Olivieri, F., Offidani, A.M., 2018. MiR-146a-5p correlates with clinical efficacy in patients with psoriasis treated with the tumour necrosis factor-alpha inhibitor adalimumab. British Journal of Dermatology 179, 787-789.

Meyers, A.K., Zhu, X., 2020. The NLRP3 Inflammasome: Metabolic Regulation and Contribution to Inflammaging. Cells 9.

Micolucci, L., Rippo, M.R., Olivieri, F., Procopio, A.D., 2017. Progress of research on microRNAs with diagnostic value in asbestos exposure: A call for method standardization. BioScience Trends 11, 105-109.

Miskowiec, D., Lipiec, P., Wierzbowska-Drabik, K., Kupczynska, K., Michalski, B., Wdowiak-Okrojek, K., Wejner-Mik, P., Kasprzak, J.D., 2016. Association between microRNA-21 concentration and lipid profile in patients with acute coronary syndrome without persistent ST-segment elevation. Pol Arch Med Wewn 126, 48-57.

Moorthy, A.K., Savinova, O.V., Ho, J.Q., Wang, V.Y., Vu, D., Ghosh, G., 2006. The 20S proteasome processes NF-kappaB1 p105 into p50 in a translation-independent manner. EMBO J 25, 1945-1956.

Morais Junior, G.S., Souza, V.C., Machado-Silva, W., Henriques, A.D., Melo Alves, A., Barbosa Morais, D., Nobrega, O.T., Brito, C.J., Dos Santos Silva, R.J., 2017. Acute strength training promotes responses in whole blood circulating levels of miR-146a among older adults with type 2 diabetes mellitus. Clin Interv Aging 12, 1443-1450.

Mulero, M.C., Wang, V.Y., Huxford, T., Ghosh, G., 2019. Genome reading by the NF-kappaB transcription factors. Nucleic Acids Res 47, 9967-9989.

Muller, M., Kuiperij, H.B., Claassen, J.A., Kusters, B., Verbeek, M.M., 2014. MicroRNAs in Alzheimer's disease: differential expression in hippocampus and cell-free cerebrospinal fluid. Neurobiol Aging 35, 152-158.

Murray, P.J., 2009. Beyond peptidoglycan for Nod2. Nat Immunol 10, 1053-1054.

Natsuka, S., Akira, S., Nishio, Y., Hashimoto, S., Sugita, T., Isshiki, H., Kishimoto, T., 1992. Macrophage differentiation-specific expression of NF-IL6, a transcription factor for interleukin-6. Blood 79, 460-466.

Newman, A.B., Walter, S., Lunetta, K.L., Garcia, M.E., Slagboom, P.E., Christensen, K., Arnold, A.M., Aspelund, T., Aulchenko, Y.S., Benjamin, E.J., Christiansen, L., D'Agostino, R.B., Sr., Fitzpatrick, A.L., Franceschini, N., Glazer, N.L., Gudnason, V., Hofman, A., Kaplan, R., Karasik, D., Kelly-Hayes, M., Kiel, D.P., Launer, L.J., Marciante, K.D., Massaro, J.M., Miljkovic, I., Nalls, M.A., Hernandez, D., Psaty, B.M., Rivadeneira, F., Rotter, J., Seshadri, S., Smith, A.V., Taylor, K.D., Tiemeier, H., Uh, H.W., Uitterlinden, A.G., Vaupel, J.W., Walston, J., Westendorp, R.G., Harris, T.B., Lumley, T., van Duijn, C.M., Murabito, J.M., 2010. A meta-analysis of four genome-wide association studies of survival to age 90 years or older: the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium. J Gerontol A Biol Sci Med Sci 65, 478-487.

Newman, J.C., Milman, S., Hashmi, S.K., Austad, S.N., Kirkland, J.L., Halter, J.B., Barzilai, N., 2016. Strategies and Challenges in Clinical Trials Targeting Human Aging. J Gerontol A Biol Sci Med Sci 71, 1424-1434.

Noren Hooten, N., Fitzpatrick, M., Wood, W.H., 3rd, De, S., Ejiogu, N., Zhang, Y., Mattison, J.A., Becker, K.G., Zonderman, A.B., Evans, M.K., 2013. Age-related changes in microRNA levels in serum. Aging (Albany NY) 5, 725-740.

Nunez Lopez, Y.O., Garufi, G., Seyhan, A.A., 2016. Altered levels of circulating cytokines and microRNAs in lean and obese individuals with prediabetes and type 2 diabetes. Mol Biosyst 13, 106-121.

Oerlemans, M.I., Mosterd, A., Dekker, M.S., de Vrey, E.A., van Mil, A., Pasterkamp, G., Doevendans, P.A., Hoes, A.W., Sluijter, J.P., 2012. Early assessment of acute coronary syndromes in the emergency department: the potential diagnostic value of circulating microRNAs. EMBO Mol Med 4, 1176-1185.

Oh, J.G., Watanabe, S., Lee, A., Gorski, P.A., Lee, P., Jeong, D., Liang, L., Liang, Y., Baccarini, A., Sahoo, S., Brown, B.D., Hajjar, R.J., Kho, C., 2018. miR-146a Suppresses SUMO1 Expression and Induces Cardiac Dysfunction in Maladaptive Hypertrophy. Circ Res 123, 673-685.

Olivieri, F., Albertini, M.C., Orciani, M., Ceka, A., Cricca, M., Procopio, A.D., Bonafè, M., 2015a. DNA damage response (DDR) and senescence: Shuttled inflamma-miRNAs on the stage of inflamm-aging. Oncotarget 6, 35509-35521.

Olivieri, F., Antonicelli, R., Lorenzi, M., D'Alessandra, Y., Lazzarini, R., Santini, G., Spazzafumo, L., Lisa, R., La Sala, L., Galeazzi, R., Recchioni, R., Testa, R., Pompilio, G., Capogrossi, M.C., Procopio, A.D., 2013a. Diagnostic potential of circulating miR-499-5p in elderly patients with acute non ST-elevation myocardial infarction. Int J Cardiol 167, 531-536.

Olivieri, F., Capri, M., Bonafe, M., Morsiani, C., Jung, H.J., Spazzafumo, L., Vina, J., Suh, Y., 2017. Circulating miRNAs and miRNA shuttles as biomarkers: Perspective trajectories of healthy and unhealthy aging. Mech Ageing Dev 165, 162-170.

Olivieri, F., Lazzarini, R., Babini, L., Prattichizzo, F., Rippo, M.R., Tiano, L., Di Nuzzo, S., Graciotti, L., Festa, R., Bruge, F., Orlando, P., Silvestri, S., Capri, M., Palma, L., Magnani, M., Franceschi, C., Littarru, G.P., Procopio, A.D., 2013b. Anti-inflammatory effect of ubiquinol-10 on young and senescent endothelial cells via miR-146a modulation. Free Radic Biol Med 63, 410-420.

Olivieri, F., Lazzarini, R., Recchioni, R., Marcheselli, F., Rippo, M.R., Di Nuzzo, S., Albertini, M.C., Graciotti, L., Babini, L., Mariotti, S., Spada, G., Abbatecola, A.M., Antonicelli, R., Franceschi, C., Procopio, A.D., 2013c. MiR-146a as marker of senescence-Associated pro-inflammatory status in cells involved in vascular remodelling. Age 35, 1157-1172.

Olivieri, F., Lazzarini, R., Recchioni, R., Marcheselli, F., Rippo, M.R., Di Nuzzo, S., Albertini, M.C., Graciotti, L., Babini, L., Mariotti, S., Spada, G., Abbatecola, A.M., Antonicelli, R., Franceschi, C., Procopio, A.D., 2013d. MiR-146a as marker of senescence-associated pro-inflammatory status in cells involved in vascular remodelling. Age (Dordr) 35, 1157-1172.

Olivieri, F., Procopio, A.D., Montgomery, R.R., 2014. Effect of aging on microRNAs and regulation of pathogen recognition receptors. Curr Opin Immunol 29, 29-37.

Olivieri, F., Rippo, M.R., Procopio, A.D., Fazioli, F., 2013e. Circulating inflamma-miRs in aging and age-related diseases. Front Genet 4, 121.

Olivieri, F., Spazzafumo, L., Bonafe, M., Recchioni, R., Prattichizzo, F., Marcheselli, F., Micolucci, L., Mensa, E., Giuliani, A., Santini, G., Gobbi, M., Lazzarini, R., Boemi, M., Testa, R., Antonicelli, R., Procopio, A.D., Bonfigli, A.R., 2015b. MiR-21-5p and miR-126a-3p levels in plasma and circulating angiogenic cells: relationship with type 2 diabetes complications. Oncotarget 6, 35372-35382.

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.

Osorio, F.G., Barcena, C., Soria-Valles, C., Ramsay, A.J., de Carlos, F., Cobo, J., Fueyo, A., Freije, J.M., Lopez-Otin, C., 2012. Nuclear lamina defects cause ATM-dependent NF-kappaB activation and link accelerated aging to a systemic inflammatory response. Genes Dev 26, 2311-2324.

Pan, Y.Q., Li, J., Li, X.W., Li, Y.C., Li, J., Lin, J.F., 2018. Effect of miR-21/TLR4/NF-kappaB pathway on myocardial apoptosis in rats with myocardial ischemia-reperfusion. Eur Rev Med Pharmacol Sci 22, 7928-7937.

Papathanasiou, I., Mourmoura, E., Balis, C., Tsezou, A., 2020. Impact of miR-SNP rs2910164 on miR-146a expression in osteoarthritic chondrocytes. Adv Med Sci 65, 78-85.

Park, M.Y., Herrmann, S.M., Saad, A., Widmer, R.J., Tang, H., Zhu, X.Y., Lerman, A., Textor, S.C., Lerman, L.O., 2015. Circulating and renal vein levels of microRNAs in patients with renal artery stenosis. Nephrol Dial Transplant 30, 480-490.

Pawelec, G., 2012. Hallmarks of human "immunosenescence": adaptation or dysregulation? Immun Ageing 9, 15.

Pawelec, G., Bronikowski, A., Cunnane, S.C., Ferrucci, L., Franceschi, C., Fulop, T., Gaudreau, P., Gladyshev, V.N., Gonos, E.S., Gorbunova, V., Kennedy, B.K., Larbi, A., Lemaitre, J.F., Liu, G.H., Maier, A.B., Morais, J.A., Nobrega, O.T., Moskalev, A., Rikkert, M.O., Seluanov, A., Senior, A.M., Ukraintseva, S., Vanhaelen, Q., Witkowski, J., Cohen, A.A., 2020. The conundrum of human immune system "senescence". Mech Ageing Dev 192, 111357.

Peng, Y., Kim, J.M., Park, H.S., Yang, A., Islam, C., Lakatta, E.G., Lin, L., 2016. AGE-RAGE signal generates a specific NF-kappaB RelA "barcode" that directs collagen I expression. Sci Rep 6, 18822.

Penzo, M., Massa, P.E., Olivotto, E., Bianchi, F., Borzi, R.M., Hanidu, A., Li, X., Li, J., Marcu, K.B., 2009. Sustained NF-kappaB activation produces a short-term cell proliferation block in conjunction with repressing effectors of cell cycle progression controlled by E2F or FoxM1. J Cell Physiol 218, 215-227.

Pfeiffer, D., Rossmanith, E., Lang, I., Falkenhagen, D., 2017. miR-146a, miR-146b, and miR-155 increase expression of IL-6 and IL-8 and support HSP10 in an In vitro sepsis model. PLoS One 12, e0179850.

Piotrowicz, K., Gasowski, J., 2020. Risk Factors for Frailty and Cardiovascular Diseases: Are They the Same? Adv Exp Med Biol 1216, 39-50.

Poli, G., Fabi, C., Bellet, M.M., Costantini, C., Nunziangeli, L., Romani, L., Brancorsini, S., 2020. Epigenetic Mechanisms of Inflammasome Regulation. Int J Mol Sci 21.

Prattichizzo, F., Bonafè, M., Olivieri, F., Franceschi, C., 2016a. Senescence associated macrophages and "macroph-aging": Are they pieces of the same puzzle? Aging 8, 3159-3160.

Prattichizzo, F., De Nigris, V., La Sala, L., Procopio, A.D., Olivieri, F., Ceriello, A., 2016b. "Inflammaging" as a Druggable Target: A Senescence-Associated Secretory Phenotype-Centered View of Type 2 Diabetes. Oxid Med Cell Longev 2016, 1810327.

Prattichizzo, F., De Nigris, V., Sabbatinelli, J., Giuliani, A., Castano, C., Parrizas, M., Crespo, I., Grimaldi, A., Baranzini, N., Spiga, R., Mancuso, E., Rippo, M.R., Procopio, A.D., Novials, A., Bonfigli, A.R., Garavelli, S., La Sala, L., Matarese, G., de Candia, P., Olivieri, F., Ceriello, A., 2021a. CD31(+) Extracellular Vesicles From Patients With Type 2 Diabetes Shuttle a miRNA Signature Associated With Cardiovascular Complications. Diabetes 70, 240-254.

Prattichizzo, F., De Nigris, V., Spiga, R., Mancuso, E., La Sala, L., Antonicelli, R., Testa, R., Procopio, A.D., Olivieri, F., Ceriello, A., 2018a. Inflammageing and metaflammation: The yin and yang of type 2 diabetes. Ageing Res Rev 41, 1-17.

Prattichizzo, F., Giuliani, A., Mensa, E., Sabbatinelli, J., De Nigris, V., Rippo, M.R., La Sala, L., Procopio, A.D., Olivieri, F., Ceriello, A., 2018b. Pleiotropic effects of metformin: Shaping the microbiome to manage type 2 diabetes and postpone ageing. Ageing Res Rev 48, 87-98.

Prattichizzo, F., Giuliani, A., Sabbatinelli, J., Matacchione, G., Ramini, D., Bonfigli, A.R., Rippo, M.R., de Candia, P., Procopio, A.D., Olivieri, F., Ceriello, A., 2020. Prevalence of residual inflammatory risk and associated clinical variables in patients with type 2 diabetes. Diabetes Obes Metab 22, 1696-1700.

Prattichizzo, F., Giuliani, A., Sabbatinelli, J., Mensà, E., De Nigris, V., La Sala, L., De Candia, P., Olivieri, F., Ceriello, A., 2019. Extracellular Vesicles Circulating in Young Organisms Promote Healthy Longevity. Journal of Extracellular Vesicles in press.

Prattichizzo, F., Matacchione, G., Giuliani, A., Sabbatinelli, J., Olivieri, F., de Candia, P., De Nigris, V., Ceriello, A., 2021b. Extracellular vesicle-shuttled miRNAs: a critical appraisal of their potential as nano-diagnostics and nano-therapeutics in type 2 diabetes mellitus and its cardiovascular complications. Theranostics 11, 1031-1045.

Prattichizzo, F., Micolucci, L., Cricca, M., De Carolis, S., Mensa, E., Ceriello, A., Procopio, A.D., Bonafe, M., Olivieri, F., 2017. Exosome-based immunomodulation during aging: A nano-perspective on inflamm-aging. Mech Ageing Dev 168, 44-53.

Prieto, L.I., Graves, S.I., Baker, D.J., 2020. Insights from In Vivo Studies of Cellular Senescence. Cells 9.

Puzianowska-Kuznicka, M., Owczarz, M., Wieczorowska-Tobis, K., Nadrowski, P., Chudek, J., Slusarczyk, P., Skalska, A., Jonas, M., Franek, E., Mossakowska, M., 2016. Interleukin-6 and C-reactive protein, successful aging, and mortality: the PolSenior study. Immun Ageing 13, 21.

Quan, X., Ji, Y., Zhang, C., Guo, X., Zhang, Y., Jia, S., Ma, W., Fan, Y., Wang, C., 2018. Circulating MiR-146a May be a Potential Biomarker of Coronary Heart Disease in Patients with Subclinical Hypothyroidism. Cell Physiol Biochem 45, 226-236.

Rabe, B., Chalaris, A., May, U., Waetzig, G.H., Seegert, D., Williams, A.S., Jones, S.A., Rose-John, S., Scheller, J., 2008. Transgenic blockade of interleukin 6 transsignaling abrogates inflammation. Blood 111, 1021-1028.

Ren, J., Zhang, J., Xu, N., Han, G., Geng, Q., Song, J., Li, S., Zhao, J., Chen, H., 2013. Signature of circulating microRNAs as potential biomarkers in vulnerable coronary artery disease. PLoS One 8, e80738.

Ren, X., Du, H., Li, Y., Yao, X., Huang, J., Li, Z., Wang, W., Li, J., Han, S., Wang, C., Huang, K., 2014. Age-related activation of MKK/p38/NF-kappaB signaling pathway in lung: from mouse to human. Exp Gerontol 57, 29-40.

Ribas, J., Ni, X., Castanares, M., Liu, M.M., Esopi, D., Yegnasubramanian, S., Rodriguez, R., Mendell, J.T., Lupold, S.E., 2012. A novel source for miR-21 expression through the alternative polyadenylation of VMP1 gene transcripts. Nucleic Acids Res 40, 6821-6833.

Riondino, S., Ferroni, P., Roselli, M., Guadagni, F., 2019. Precision medicine in the ageing world: The role of biospecimen sciences. Int J Biol Markers 34, 3-5.

Robinson, S., Follo, M., Haenel, D., Mauler, M., Stallmann, D., Heger, L.A., Helbing, T., Duerschmied, D., Peter, K., Bode, C., Ahrens, I., Hortmann, M., 2018. Chip-based digital PCR as a novel detection method for quantifying microRNAs in acute myocardial infarction patients. Acta Pharmacol Sin 39, 1217-1227.

Rodier, F., Coppe, J.P., Patil, C.K., Hoeijmakers, W.A., Munoz, D.P., Raza, S.R., Freund, A., Campeau, E., Davalos, A.R., Campisi, J., 2009. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol 11, 973-979.

Rosa, M., Chignon, A., Li, Z., Boulanger, M.C., Arsenault, B.J., Bosse, Y., Theriault, S., Mathieu, P., 2019. A Mendelian randomization study of IL6 signaling in cardiovascular diseases, immune-related disorders and longevity. NPJ Genom Med 4, 23.

Rose, G., Romeo, G., Dato, S., Crocco, P., Bruni, A.C., Hervonen, A., Majamaa, K., Sevini, F., Franceschi, C., Passarino, G., Consortium, G.E.o.H.A.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.

Runtsch, M.C., Nelson, M.C., Lee, S.H., Voth, W., Alexander, M., Hu, R., Wallace, J., Petersen, C., Panic, V., Villanueva, C.J., Evason, K.J., Bauer, K.M., Mosbruger, T., Boudina, S., Bronner, M., Round, J.L., Drummond, M.J.,

O'Connell, R.M., 2019. Anti-inflammatory microRNA-146a protects mice from diet-induced metabolic disease. PLoS Genet 15, e1007970.
Rusanova, I., Diaz-Casado, M.E., Fernandez-Ortiz, M., Aranda-Martinez, P., Guerra-Librero, A., Garcia-Garcia, F.J., Escames, G., Manas, L., Acuna-Castroviejo, D., 2018. Analysis of Plasma MicroRNAs as Predictors and Biomarkers of Aging and Frailty in Humans. Oxid Med Cell Longev 2018, 7671850.
Sabbatinelli, J., Giuliani, A., Matacchione, G., Latini, S., Laprovitera, N., Pomponio, G., Ferrarini, A., Svegliati Baroni, S., Pavani, M., Moretti, M., Gabrielli, A., Procopio, A.D., Ferracin, M., Bonafe, M., Olivieri, F., 2021. Decreased serum levels of the inflammaging marker miR-146a are associated with clinical non-response to tocilizumab in COVID-19 patients. Mech Ageing Dev 193, 111413.
Sabre, L., Maddison, P., Sadalage, G., Ambrose, P.A., Punga, A.R., 2018. Circulating microRNA miR-21-5p, miR-150-5p and miR-30e-5p correlate with clinical status in late onset myasthenia gravis. J Neuroimmunol 321, 164-170.
Saferding, V., Hofmann, M., Brunner, J.S., Niederreiter, B., Timmen, M., Magilnick, N., Hayer, S., Heller, G., Steiner, G., Stange, R., Boldin, M., Schabbauer, G., Weigl, M., Hackl, M., Grillari, J., Smolen, J.S., Bluml, S., 2020. microRNA-146a controls age-related bone loss. Aging Cell 19, e13244.
Saferding, V., Puchner, A., Goncalves-Alves, E., Hofmann, M., Bonelli, M., Brunner, J.S., Sahin, E., Niederreiter, B., Hayer, S., Kiener, H.P., Einwallner, E., Nehmar, R., Carapito, R., Georgel, P., Koenders, M.I., Boldin, M., Schabbauer, G., Kurowska-Stolarska, M., Steiner, G., Smolen, J.S., Redlich, K., Bluml, S., 2017. MicroRNA-146a governs fibroblast activation and joint pathology in arthritis. J Autoimmun 82, 74-84.
Salminen, A., Huuskonen, J., Ojala, J., Kauppinen, A., Kaarniranta, K., Suuronen, T., 2008. Activation of innate immunity system during aging: NF-kB signaling is the molecular culprit of inflamm-aging. Ageing Res Rev 7, 83-105.
Salminen, A., Kauppinen, A., Kaarniranta, K., 2012. Emerging role of NF-kappaB signaling in the induction of senescence-associated secretory phenotype (SASP). Cell Signal 24, 835-845.
Scheidereit, C., 2006. IkappaB kinase complexes: gateways to NF-kappaB activation and transcription. Oncogene 25, 6685-6705.
Scheller, J., Chalaris, A., Schmidt-Arras, D., Rose-John, S., 2011. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 1813, 878-888.
Schultz, M.B., Kane, A.E., Mitchell, S.J., MacArthur, M.R., Warner, E., Vogel, D.S., Mitchell, J.R., Howlett, S.E., Bonkowski, M.S., Sinclair, D.A., 2020. Age and life expectancy clocks based on machine learning analysis of mouse frailty. Nat Commun 11, 4618.
Sebastian, C., Espia, M., Serra, M., Celada, A., Lloberas, J., 2005. MacrophAging: a cellular and molecular review. Immunobiology 210, 121-126.
Sebastian-Valverde, M., Pasinetti, G.M., 2020. The NLRP3 Inflammasome as a Critical Actor in the Inflammaging Process. Cells 9.
Senftleben, U., Cao, Y., Xiao, G., Greten, F.R., Krahn, G., Bonizzi, G., Chen, Y., Hu, Y., Fong, A., Sun, S.C., Karin, M., 2001. Activation by IKKalpha of a second, evolutionary conserved, NF-kappa B signaling pathway. Science 293, 1495-1499.
Servais, F.A., Kirchmeyer, M., Hamdorf, M., Minoungou, N.W.E., Rose-John, S., Kreis, S., Haan, C., Behrmann, I., 2019. Modulation of the IL-6-Signaling Pathway in Liver Cells by miRNAs Targeting gp130, JAK1, and/or STAT3. Mol Ther Nucleic Acids 16, 419-433.
Seyhan, A.A., Nunez Lopez, Y.O., Xie, H., Yi, F., Mathews, C., Pasarica, M., Pratley, R.E., 2016. Pancreas-enriched miRNAs are altered in the circulation of subjects with diabetes: a pilot cross-sectional study. Sci Rep 6, 31479.
Sheedy, F.J., 2015. Turning 21: Induction of miR-21 as a Key Switch in the Inflammatory Response. Front Immunol 6, 19.
Sheedy, F.J., Palsson-McDermott, E., Hennessy, E.J., Martin, C., O'Leary, J.J., Ruan, Q., Johnson, D.S., Chen, Y., O'Neill, L.A., 2010. Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. Nat Immunol 11, 141-147.
Shin, V.Y., Jin, H., Ng, E.K., Cheng, A.S., Chong, W.W., Wong, C.Y., Leung, W.K., Sung, J.J., Chu, K.M., 2011. NF-kappaB targets miR-16 and miR-21 in gastric cancer: involvement of prostaglandin E receptors. Carcinogenesis 32, 240-245.
Shukla, S., Bjerke, G.A., Muhlrad, D., Yi, R., Parker, R., 2019. The RNase PARN Controls the Levels of Specific miRNAs that Contribute to p53 Regulation. Mol Cell 73, 1204-1216 e1204.
Singh, N., Heggermont, W., Fieuws, S., Vanhaecke, J., Van Cleemput, J., De Geest, B., 2015. Endothelium-enriched microRNAs as diagnostic biomarkers for cardiac allograft vasculopathy. J Heart Lung Transplant 34, 1376-1384.
Smigielska-Czepiel, K., van den Berg, A., Jellema, P., van der Lei, R.J., Bijzet, J., Kluiver, J., Boots, A.M., Brouwer, E., Kroesen, B.J., 2014. Comprehensive analysis of miRNA expression in T-cell subsets of rheumatoid arthritis patients reveals defined signatures of naive and memory Tregs. Genes Immun 15, 115-125.
Son, Y.H., Jeong, Y.T., Lee, K.A., Choi, K.H., Kim, S.M., Rhim, B.Y., Kim, K., 2008. Roles of MAPK and NF-kappaB in interleukin-6 induction by lipopolysaccharide in vascular smooth muscle cells. J Cardiovasc Pharmacol 51, 71-77.
Song, P., An, J., Zou, M.H., 2020a. Immune Clearance of Senescent Cells to Combat Ageing and Chronic Diseases. Cells 9.

Song, S., Lam, E.W., Tchkonia, T., Kirkland, J.L., Sun, Y., 2020b. Senescent Cells: Emerging Targets for Human Aging and Age-Related Diseases. Trends Biochem Sci 45, 578-592.
Song, Y., Dou, H., Li, X., Zhao, X., Li, Y., Liu, D., Ji, J., Liu, F., Ding, L., Ni, Y., Hou, Y., 2017. Exosomal miR-146a Contributes to the Enhanced Therapeutic Efficacy of Interleukin-1beta-Primed Mesenchymal Stem Cells Against Sepsis. Stem Cells 35, 1208-1221.
Sorensen, S.S., Nygaard, A.B., Christensen, T., 2016. miRNA expression profiles in cerebrospinal fluid and blood of patients with Alzheimer's disease and other types of dementia - an exploratory study. Transl Neurodegener 5, 6.
Stenholm, S., Metter, E.J., Roth, G.S., Ingram, D.K., Mattison, J.A., Taub, D.D., Ferrucci, L., 2011. Relationship between plasma ghrelin, insulin, leptin, interleukin 6, adiponectin, testosterone and longevity in the Baltimore Longitudinal Study of Aging. Aging Clin Exp Res 23, 153-158.
Storci, G., Bacalini, M.G., Bonifazi, F., Garagnani, P., De Carolis, S., Salvioli, S., Olivieri, F., Bonafe, M., 2020. Ribosomal DNA instability: An evolutionary conserved fuel for inflammaging. Ageing Res Rev 58, 101018.
Storci, G., De Carolis, S., Olivieri, F., Bonafe, M., 2018. Changes in the biochemical taste of cytoplasmic and cell-free DNA are major fuels for inflamm-aging. Semin Immunol 40, 6-16.
Strowig, T., Henao-Mejia, J., Elinav, E., Flavell, R., 2012. Inflammasomes in health and disease. Nature 481, 278-286.
Su, Y.L., Wang, X., Mann, M., Adamus, T.P., Wang, D., Moreira, D.F., Zhang, Z., Ouyang, C., He, X., Zhang, B., Swiderski, P.M., Forman, S.J., Baltimore, D., Li, L., Marcucci, G., Boldin, M.P., Kortylewski, M., 2020. Myeloid cell-targeted miR-146a mimic inhibits NF-kappaB-driven inflammation and leukemia progression in vivo. Blood 135, 167-180.
Sugimoto, M.A., Sousa, L.P., Pinho, V., Perretti, M., Teixeira, M.M., 2016. Resolution of Inflammation: What Controls Its Onset? Front Immunol 7, 160.
Sulli, G., Di Micco, R., d'Adda di Fagagna, F., 2012. Crosstalk between chromatin state and DNA damage response in cellular senescence and cancer. Nat Rev Cancer 12, 709-720.
Sun, S.C., 2017. The non-canonical NF-kappaB pathway in immunity and inflammation. Nat Rev Immunol 17, 545-558.
Szemraj, M., Bielecka-Kowalska, A., Oszajca, K., Krajewska, M., Gos, R., Jurowski, P., Kowalski, M., Szemraj, J., 2015. Serum MicroRNAs as Potential Biomarkers of AMD. Med Sci Monit 21, 2734-2742.
Taganov, K.D., Boldin, M.P., Chang, K.J., Baltimore, D., 2006. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci U S A 103, 12481-12486.
Tanaka, T., Basisty, N., Fantoni, G., Candia, J., Moore, A.Z., Biancotto, A., Schilling, B., Bandinelli, S., Ferrucci, L., 2020. Plasma proteomic biomarker signature of age predicts health and life span. Elife 9.
Tanaka, T., Kishimoto, T., 2014. The biology and medical implications of interleukin-6. Cancer Immunol Res 2, 288-294.
Tchkonia, T., Zhu, Y., van Deursen, J., Campisi, J., Kirkland, J.L., 2013. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest 123, 966-972.
Telonis, A.G., Loher, P., Jing, Y., Londin, E., Rigoutsos, I., 2015. Beyond the one-locus-one-miRNA paradigm: microRNA isoforms enable deeper insights into breast cancer heterogeneity. Nucleic Acids Res 43, 9158-9175.
Teodori, L., Petrignani, I., Giuliani, A., Prattichizzo, F., Gurău, F., Matacchione, G., Olivieri, F., Coppari, S., Albertini, M.C., 2019. Inflamm-aging microRNAs may integrate signals from food and gut microbiota by modulating common signalling pathways. Mech Ageing Dev 182, 111127.
Thum, T., Gross, C., Fiedler, J., Fischer, T., Kissler, S., Bussen, M., Galuppo, P., Just, S., Rottbauer, W., Frantz, S., Castoldi, M., Soutschek, J., Koteliansky, V., Rosenwald, A., Basson, M.A., Licht, J.D., Pena, J.T., Rouhanifard, S.H., Muckenthaler, M.U., Tuschl, T., Martin, G.R., Bauersachs, J., Engelhardt, S., 2008. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature 456, 980-984.
Tilstra, J.S., Robinson, A.R., Wang, J., Gregg, S.Q., Clauson, C.L., Reay, D.P., Nasto, L.A., St Croix, C.M., Usas, A., Vo, N., Huard, J., Clemens, P.R., Stolz, D.B., Guttridge, D.C., Watkins, S.C., Garinis, G.A., Wang, Y., Niedernhofer, L.J., Robbins, P.D., 2012. NF-kappaB inhibition delays DNA damage-induced senescence and aging in mice. J Clin Invest 122, 2601-2612.
Torri, A., Carpi, D., Bulgheroni, E., Crosti, M.C., Moro, M., Gruarin, P., Rossi, R.L., Rossetti, G., Di Vizio, D., Hoxha, M., Bollati, V., Gagliani, C., Tacchetti, C., Paroni, M., Geginat, J., Corti, L., Venegoni, L., Berti, E., Pagani, M., Matarese, G., Abrignani, S., de Candia, P., 2017. Extracellular MicroRNA Signature of Human Helper T Cell Subsets in Health and Autoimmunity. J Biol Chem 292, 2903-2915.
Tuttle, C.S.L., Waaijer, M.E.C., Slee-Valentijn, M.S., Stijnen, T., Westendorp, R., Maier, A.B., 2020. Cellular senescence and chronological age in various human tissues: A systematic review and meta-analysis. Aging Cell 19, e13083.
van der Kwast, R., Woudenberg, T., Quax, P.H.A., Nossent, A.Y., 2020. MicroRNA-411 and Its 5'-IsomiR Have Distinct Targets and Functions and Are Differentially Regulated in the Vasculature under Ischemia. Mol Ther 28, 157-170.
Vande Walle, L., Van Opdenbosch, N., Jacques, P., Fossoul, A., Verheugen, E., Vogel, P., Beyaert, R., Elewaut, D., Kanneganti, T.D., van Loo, G., Lamkanfi, M., 2014. Negative regulation of the NLRP3 inflammasome by A20 protects against arthritis. Nature 512, 69-73.

Velle-Forbord, T., Eidlaug, M., Debik, J., Saether, J.C., Follestad, T., Nauman, J., Gigante, B., Rosjo, H., Omland, T., Langaas, M., Bye, A., 2019. Circulating microRNAs as predictive biomarkers of myocardial infarction: Evidence from the HUNT study. Atherosclerosis 289, 1-7.

Villar, A.V., Garcia, R., Merino, D., Llano, M., Cobo, M., Montalvo, C., Martin-Duran, R., Hurle, M.A., Nistal, J.F., 2013. Myocardial and circulating levels of microRNA-21 reflect left ventricular fibrosis in aortic stenosis patients. Int J Cardiol 167, 2875-2881.

Vitsios, D.M., Davis, M.P., van Dongen, S., Enright, A.J., 2017. Large-scale analysis of microRNA expression, epi-transcriptomic features and biogenesis. Nucleic Acids Res 45, 1079-1090.

Walford, R.L., 1964. The Immunologic Theory of Aging. Gerontologist 4, 195-197.

Wang, F., Long, G., Zhao, C., Li, H., Chaugai, S., Wang, Y., Chen, C., Wang, D.W., 2014. Atherosclerosis-related circulating miRNAs as novel and sensitive predictors for acute myocardial infarction. PLoS One 9, e105734.

Wang, J., Yan, Y., Song, D., Liu, B., 2016. Reduced Plasma miR-146a Is a Predictor of Poor Coronary Collateral Circulation in Patients with Coronary Artery Disease. Biomed Res Int 2016, 4285942.

Wang, X., Sundquist, K., Svensson, P.J., Rastkhani, H., Palmer, K., Memon, A.A., Sundquist, J., Zoller, B., 2019. Association of recurrent venous thromboembolism and circulating microRNAs. Clin Epigenetics 11, 28.

Watanabe, K., Narumi, T., Watanabe, T., Otaki, Y., Takahashi, T., Aono, T., Goto, J., Toshima, T., Sugai, T., Wanezaki, M., Kutsuzawa, D., Kato, S., Tamura, H., Nishiyama, S., Takahashi, H., Arimoto, T., Shishido, T., Watanabe, M., 2020. The association between microRNA-21 and hypertension-induced cardiac remodeling. PLoS One 15, e0226053.

Wei, W.J., Wang, Y.L., Li, D.S., Wang, Y., Wang, X.F., Zhu, Y.X., Yang, Y.J., Wang, Z.Y., Ma, Y.Y., Wu, Y., Jin, L., Ji, Q.H., Wang, J.C., 2013. Association between the rs2910164 polymorphism in pre-Mir-146a sequence and thyroid carcinogenesis. PLoS One 8, e56638.

West, A.P., Brodsky, I.E., Rahner, C., Woo, D.K., Erdjument-Bromage, H., Tempst, P., Walsh, M.C., Choi, Y., Shadel, G.S., Ghosh, S., 2011. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. Nature 472, 476-480.

Wirasinha, R.C., Davies, A.R., Srivastava, M., Sheridan, J.M., Sng, X.Y.X., Delmonte, O.M., Dobbs, K., Loh, K.L., Miosge, L.A., Lee, C.E., Chand, R., Chan, A., Yap, J.Y., Keller, M.D., Chen, K., Rossjohn, J., La Gruta, N.L., Vinuesa, C.G., Reid, H.H., Lionakis, M.S., Notarangelo, L.D., Gray, D.H.D., Goodnow, C.C., Cook, M.C., Daley, S.R., 2021. Nfkb2 variants reveal a p100-degradation threshold that defines autoimmune susceptibility. J Exp Med 218.

Wisdom, R., 1999. AP-1: one switch for many signals. Exp Cell Res 253, 180-185.

Wu, J., Fan, C.L., Ma, L.J., Liu, T., Wang, C., Song, J.X., Lv, Q.S., Pan, H., Zhang, C.N., Wang, J.J., 2017a. Distinctive expression signatures of serum microRNAs in ischaemic stroke and transient ischaemic attack patients. Thromb Haemost 117, 992-1001.

Wu, Y., Xu, J., Xu, J., Cheng, J., Jiao, D., Zhou, C., Dai, Y., Chen, Q., 2017b. Lower Serum Levels of miR-29c-3p and miR-19b-3p as Biomarkers for Alzheimer's Disease. Tohoku J Exp Med 242, 129-136.

Xu, T., Zhou, Q., Che, L., Das, S., Wang, L., Jiang, J., Li, G., Xu, J., Yao, J., Wang, H., Dai, Y., Xiao, J., 2016. Circulating miR-21, miR-378, and miR-940 increase in response to an acute exhaustive exercise in chronic heart failure patients. Oncotarget 7, 12414-12425.

Xu, T., Zhu, Y., Wei, Q.K., Yuan, Y., Zhou, F., Ge, Y.Y., Yang, J.R., Su, H., Zhuang, S.M., 2008. A functional polymorphism in the miR-146a gene is associated with the risk for hepatocellular carcinoma. Carcinogenesis 29, 2126-2131.

Xue, S., Zhu, W., Liu, D., Su, Z., Zhang, L., Chang, Q., Li, P., 2019a. Circulating miR-26a-1, miR-146a and miR-199a-1 are potential candidate biomarkers for acute myocardial infarction. Mol Med 25, 18.

Xue, Z., Xi, Q., Liu, H., Guo, X., Zhang, J., Zhang, Z., Li, Y., Yang, G., Zhou, D., Yang, H., Zhang, L., Zhang, Q., Gu, C., Yang, J., Da, Y., Yao, Z., Duo, S., Zhang, R., 2019b. miR-21 promotes NLRP3 inflammasome activation to mediate pyroptosis and endotoxic shock. Cell Death Dis 10, 461.

Yamada, H., Suzuki, K., Ichino, N., Ando, Y., Sawada, A., Osakabe, K., Sugimoto, K., Ohashi, K., Teradaira, R., Inoue, T., Hamajima, N., Hashimoto, S., 2013. Associations between circulating microRNAs (miR-21, miR-34a, miR-122 and miR-451) and non-alcoholic fatty liver. Clin Chim Acta 424, 99-103.

Yang, W.L., Wang, J., Chan, C.H., Lee, S.W., Campos, A.D., Lamothe, B., Hur, L., Grabiner, B.C., Lin, X., Darnay, B.G., Lin, H.K., 2009. The E3 ligase TRAF6 regulates Akt ubiquitination and activation. Science 325, 1134-1138.

Yang, X., Du, X., Ma, K., Li, G., Liu, Z., Rong, W., Miao, H., Zhu, F., Cui, Q., Wu, S., Li, Y., Du, J., 2021. Circulating miRNAs Related to Long-term Adverse Cardiovascular Events in STEMI Patients: A Nested Case-Control Study. Can J Cardiol 37, 77-85.

Yildirim, E., Ermis, E., Allahverdiyev, S., Ucar, H., Yavuzer, S., Cengiz, M., 2019. Circulating miR-21 levels in hypertensive patients with asymptomatic organ damage. Medicine (Baltimore) 98, e17297.

Youm, Y.H., Grant, R.W., McCabe, L.R., Albarado, D.C., Nguyen, K.Y., Ravussin, A., Pistell, P., Newman, S., Carter, R., Laque, A., Munzberg, H., Rosen, C.J., Ingram, D.K., Salbaum, J.M., Dixit, V.D., 2013. Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. Cell Metab 18, 519-532.

Youm, Y.H., Kanneganti, T.D., Vandanmagsar, B., Zhu, X., Ravussin, A., Adijiang, A., Owen, J.S., Thomas, M.J., Francis, J., Parks, J.S., Dixit, V.D., 2012. The Nlrp3 inflammasome promotes age-related thymic demise and immunosenescence. Cell Rep 1, 56-68.

Yu, Q., Katlinskaya, Y.V., Carbone, C.J., Zhao, B., Katlinski, K.V., Zheng, H., Guha, M., Li, N., Chen, Q., Yang, T., Lengner, C.J., Greenberg, R.A., Johnson, F.B., Fuchs, S.Y., 2015. DNA-damage-induced type I interferon promotes senescence and inhibits stem cell function. Cell Rep 11, 785-797.

Zampetaki, A., Kiechl, S., Drozdov, I., Willeit, P., Mayr, U., Prokopi, M., Mayr, A., Weger, S., Oberhollenzer, F., Bonora, E., Shah, A., Willeit, J., Mayr, M., 2010. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ Res 107, 810-817.

Zhang, B., Yao, Y., Sun, Q.F., Liu, S.Q., Jing, B., Yuan, C., Liu, X.Y., Jiao, T., Li, H.C., Wang, H.Y., 2017a. Circulating mircoRNA-21 as a predictor for vascular restenosis after interventional therapy in patients with lower extremity arterial occlusive disease. Biosci Rep 37.

Zhang, J., Zhang, Z.G., Lu, M., Zhang, Y., Shang, X., Chopp, M., 2019. MiR-146a promotes oligodendrocyte progenitor cell differentiation and enhances remyelination in a model of experimental autoimmune encephalomyelitis. Neurobiol Dis 125, 154-162.

Zhang, Q.B., Qing, Y.F., Yin, C.C., Zhou, L., Liu, X.S., Mi, Q.S., Zhou, J.G., 2018a. Mice with miR-146a deficiency develop severe gouty arthritis via dysregulation of TRAF 6, IRAK 1 and NALP3 inflammasome. Arthritis Res Ther 20, 45.

Zhang, X., Wang, C., Zhao, J., Xu, J., Geng, Y., Dai, L., Huang, Y., Fu, S.C., Dai, K., Zhang, X., 2017b. miR-146a facilitates osteoarthritis by regulating cartilage homeostasis via targeting Camk2d and Ppp3r2. Cell Death Dis 8, e2734.

Zhang, Z.J., Guo, J.S., Li, S.S., Wu, X.B., Cao, D.L., Jiang, B.C., Jing, P.B., Bai, X.Q., Li, C.H., Wu, Z.H., Lu, Y., Gao, Y.J., 2018b. TLR8 and its endogenous ligand miR-21 contribute to neuropathic pain in murine DRG. J Exp Med 215, 3019-3037.

Zhao, J.L., Rao, D.S., Boldin, M.P., Taganov, K.D., O'Connell, R.M., Baltimore, D., 2011. NF-kappaB dysregulation in microRNA-146a-deficient mice drives the development of myeloid malignancies. Proc Natl Acad Sci U S A 108, 9184-9189.

Zhao, J.L., Rao, D.S., O'Connell, R.M., Garcia-Flores, Y., Baltimore, D., 2013. MicroRNA-146a acts as a guardian of the quality and longevity of hematopoietic stem cells in mice. Elife 2, e00537.

Zhou, Q., Maleck, C., von Ungern-Sternberg, S.N.I., Neupane, B., Heinzmann, D., Marquardt, J., Duckheim, M., Scheckenbach, C., Stimpfle, F., Gawaz, M., Schreieck, J., Seizer, P., Gramlich, M., 2018a. Circulating MicroRNA-21 Correlates With Left Atrial Low-Voltage Areas and Is Associated With Procedure Outcome in Patients Undergoing Atrial Fibrillation Ablation. Circ Arrhythm Electrophysiol 11, e006242.

Zhou, W., Su, L., Duan, X., Chen, X., Hays, A., Upadhyayula, S., Shivde, J., Wang, H., Li, Y., Huang, D., Liang, S., 2018b. MicroRNA-21 down-regulates inflammation and inhibits periodontitis. Mol Immunol 101, 608-614.

Zinszner, H., Kuroda, M., Wang, X., Batchvarova, N., Lightfoot, R.T., Remotti, H., Stevens, J.L., Ron, D., 1998. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev 12, 982-995.

Table 1. Summary of the studies evaluating miR-146a in different body fluids as a biomarker for type 2 diabetes, cardiovascular diseases, and neurodegenerative disorders.

Disease	Type of Sample	Study population	Levels in disease	Detailed findings	Ref.
Type 2 diabetes					
T2D	plasma	T2DM (n=188) healthy controls (n=144)	↓	- Lower in diabetic patients than in age-matched CTR - Overexpressed in T2DM patients treated with metformin	(Mensà et al., 2019)
Impaired glucose tolerance and T2D with and without complications	serum	pre-diabetic (n=16) T2DM without complications (n = 54) T2DM with presence of nephropathy (n = 18), diabetic foot (n = 3) or CVD (n = 7) healthy controls (n = 35).	↓ (pre-diabetes)	- Decreased in pre-diabetic patients compared to control group - Increased in T2DM patients compared to pre-diabetic patients - Decreased in T2DM patients with nephropathy and diabetic compared to T2DM patients without complications	(Garcia-Jacobo et al., 2019)
T2D	plasma	T2DM (n=30) healthy controls (n=30)	↓	- Decreased in T2DM compared to healthy participants - rs2910164-C allele is associated with reduced expression of miR-146a	(Alipoor et al., 2018)
Impaired Glucose tolerance and T2D	serum	prediabetes (n=21,10 lean + 11 with obesity) T2DM (n=17,2 lean + 15 with obesity) healthy controls (11 lean + 9 with obesity)	=	- Associated with diabetes independent of obesity and BMI - Increased in the T2DM group compared to the prediabetes group	(Nunez Lopez et al., 2016)
T2D	blood	T2DM (n=13) healthy controls (n=10)	↑	Increased expression in diabetic compared with nondiabetic patients, after a strength training intervention	(Morais Junior et al., 2017)
Cardiovascular diseases					
Coronary heart disease (CHD) with subclinical hypothyroidism (SCH) or without SCH	plasma	CHD+SCH (n=60) CHD (n=73) healthy controls (n=59)	↑	- Increased levels in CHD and CHD+SCH patients - Highest in CHD+SCH patients showing the largest increase - AUC=0.779 for the diagnosis of CHD	(Quan et al., 2018)

Acute myocardial infraction (MI)	plasma	LVR (n = 56) Non-LVR (n = 142)	↑	Higher in patients with LVR	(Liu et al., 2015)
Acute coronary syndrome (ACS) with or without chronic periodontitis (CP)	serum	ACS without CP (n=66) ACS with CP (n=66) CP only (n=66) healthy controls (n=66)	↑	Upregulated in all disease groups compared to controls	(Bagavad Gita et al., 2019)
Ischemic stroke (IS)		IS (n=60, acute or sub-acute phase) healthy controls (n=30)	↑/↓	- Decreased in acute IS compared with healthy individuals - Downregulated in acute IS but upregulated in subacute IS	(Li et al., 2015b)
Premature acute coronary syndrome (ACS), with or without a history of preeclampsia (PE)	plasma	ACS with prior PE (n = 30) ACS with prior normotensive pregnancy (n = 146)	=	Decreased in ACS women with prior PE compared to ACS women with prior normotensive pregnancy	(Dayan et al., 2018)
Coronary artery disease (CAD) with poor or good Coronary Collateral Circulation (CCC)	plasma	patients with poor CCC (n=34) patients with good CCC (n=34) healthy control subjects (n=34)	↑/↓	- Increased in CAD patients with good CCC - Decreased in patients with poor CCC	(Wang et al., 2016)
Acute coronary syndromes (ACS) witj unstable angina (UA) and non-ST-elevation myocardial infarction (NSTEMI)	serum	ACS (n=106; UA, n = 24 and NSTEMI, n = 82) non-ACS (n=226)	↑	- Increased in ACS patients - Increased in UA and STEMI - Highest in NSTEMI patients	(Oerlemans et al., 2012)
Hypertension	plasma	hypertensive (n=15) normotensive (n=15)	↓	- Lower in the hypertensive vs normotensive group - Significantly related to both systolic and diastolic blood pressure	(Hijmans et al., 2018)
Acute myocardial infarction (AMI)	plasma	AMI (n=31) controls (n=27)	↑	- Increased in AMI patients - High diagnostic efficiency for predicting AMI by ROC analysis	(Xue et al., 2019a)

Dilated cardiomyopathy (DCM)	plasma	DCM (n=45) controls (n=39)	=	No difference between DCM and control groups	(Fan et al., 2013)
Heart failure	plasma (exosome)	heart failure (n=40) controls (n=20)	↑	- Higher circulating exosomal miR-146a/miR-16 ratio in heart failure patients - TNFα and GM-CSF increased miR-146a but not miR-486 in the cardiomyocyte cell line H9C2	(Beg et al., 2017)
Acute myocardial infarction (AMI)	plasma	AMI (n=31) controls (n=27)	↑	Considerable diagnostic efficiency for predicting AMI	(Xue et al., 2019a)
Acute ischemic stroke (AIS)	blood	AIS (n=30) Healthy controls (n=30)	↓	- Downregulated in AIS - Fbxl10 was identified as a target of miR-146a - Upregulation of Fbxl10 likely protects neurons from ischemic death	(Li et al., 2017)
Hypertrophic cardiomyopathy (HCM)	plasma	HCM (n=8; T1 < 470 ms, n=4; T1 ≥ 470 ms, n=4) healthy controls (n=4)	↑	Upregulated in T1 ≥ 470 ms compared to T1 < 470 ms and controls	(Fang et al., 2015)
Neurodegenerative diseases					
Alzheimer's disease (AD), mild cognitive impairment (MCI) and vascular dementia (VD)	serum	AD (n=127) MCI (n=30) VD (n=30) non-dementia controls (NDC, n = 123)	↓	- Decreased in AD patient serum compared with controls - Higher in MCI compared to controls	(Dong et al., 2015)
Alzheimer's disease	serum	AD (n=20) healthy controls (n=20)	↑	Higher in AD patients compared with control subjects	(Wu et al., 2017b)

Alzheimer's disease	plasma	AD (n=116) and healthy controls (n=40) ≥ 65 yrs	=	Significant positive correlation between CRP and miR-146a in AD patients	(Giuliani et al., 2021)
Alzheimer's disease	plasma CSF	AD (n=10) healthy controls (n=10)	↓	Plasma and CSF miR-146a levels in AD patients were significantly lower than in control subjects	(Kiko et al., 2014)
Alzheimer's disease	plasma CSF	AD (n=20) healthy controls (n=20)	↓ (plasma) ↑ (CSF)	- Lower plasma level in AD group compared to control group - Higher CSF miR-146a in AD compared to healthy controls	(Gong and Sun, 2020)
Alzheimer's disease	CSF	AD (n = 22) healthy controls (n = 28)	↑	- Upregulated in CSF of AD patients - Significant inverse correlation with tau and Aβ1–42	(Denk et al., 2015)
Alzheimer's disease	CSF	AD (n = 20) Healthy controls (n = 22)	↓	Low levels of miR-146a in CSF were associated with AD	(Muller et al., 2014)

CSF, cerebrospinal fluid.

Table 2. Summary of the studies evaluating miR-21-5p in different body fluids as a biomarker for type 2 diabetes, cardiovascular diseases, and neurodegenerative disorders.

Disease	Type of sample	Study population	Levels in disease	Detailed findings	Ref.
Type 2 diabetes					
Heart failure and stable CAD in T2D	serum	T2D (n=45) T2D+CAD (n=45) T2D+HF (n=45) healthy controls (n=45)	↑	- Overexpression in patients with DM, CAD + DM, and HF + DM compared with controls - Higher in the HF + DM group compared to the CAD + DM group	(Al-Hayali et al., 2019)
Impaired glucose tolerance (IGT) and T2D	plasma	IGT (n=43) newly diagnosed, drug naïve T2D (T2D, n = 27) NGT (n=39)	↑	- Overexpression in IGT and in T2D compared to NGT subjects - Positive correlation with glycaemic parameters - High and accurate diagnostic performance	(La Sala et al., 2019)
IGT and T2D	plasma	21 with prediabetes (10 lean + 11 with obesity) 17 with T2D (2 lean + 15 with obesity) healthy controls (11 lean + 9 with obesity)	↑	- Increased in the T2D group vs. prediabetes and healthy control groups - Associated with diabetes independent of obesity and BMI status	(Nunez Lopez et al., 2016)
Diabetes (multiple etiologies)	plasma	prediabetes (n = 12) type 2 diabetes (T2D, n = 31) latent autoimmune diabetes of adults (LADA, n = 6) type 1 diabetes (T1D, n = 16) healthy control subjects (n = 27).	↑	- Increased in T2D and T1D vs. healthy controls - No significant difference among diabetes subtypes	(Seyhan et al., 2016)
Diabetic nephropathy (DN)	serum	T2D subjects categorized by Albumin Creatinine Ratio: normal albuminuria (n=11) microalbuminuria (n=17) overt proteinuria (n=21)	N/A	- Increased in patients with overt proteinuria compared with the patients with normal or micro- albuminuria. - Increased in subjects with lower eGFR	(Chien et al., 2016)
T2D with (T2D-C) or without (T2D-NC) compications	plasma	T2D (N=193) T2D-NC (n=76) T2D-C (n=117) healthy subjects (n=107)	↓	- Significant decline from CTR to T2D-NC and T2D-C - Higher in MACE compared to other complications	(Olivieri et al., 2015b)
T2D	plasma	T2D (N=80) healthy controls (n=80)	↓		(Zampetaki et al., 2010)
Cardiovascular diseases					

Recurrent CVD after ST-segment-elevation myocardial infarction (STEMI)	serum	MACE (n=70) non-MACE (n=140) healthy control (n=20)	↓	- Downregulated in MACE and non-MACE compared to healthy controls - Lower in MACE vs. non-MACE - Negative association with incident primary composite outcomes	(Yang et al., 2021)
Heart failure (HF)	plasma	HF (N=62) healthy controls (n=62)	↑	High diagnostic accuracy	(Ding et al., 2020a)
Hypertensive heart disease (HHD)	serum	HHD (n=10) controls (n=10)	↑	Correlation with serum myocardial fibrotic markers	(Watanabe et al., 2020)
Hypertension (HT) with asymptomatic organ damage (AOD)	plasma	HT (n=16) HT+AOD (n=16) healthy controls (n=32)	↑	- Upregulated in HT vs. controls - Upregulated in HT+AOD vs. HT - Correlations with clinical systolic and diastolic blood pressure, microalbuminuria, C-reactive protein, and carotid intima-media thickness	(Yildirim et al., 2019)
Myocardial infarction (MI)	serum	10-year observation period of 195 healthy participants: fatal MI (n = 36) non-fatal MI (n = 60) controls (n = 99) remained healthy	↑	Increasing serum miR-21-5p levels add significantly to Framingham risk score as a predictor of 10-year risk of MI	(Velle-Forbord et al., 2019)
Venous thromboembolism (VTE)	plasma	recurrent VTE (n=39) no-recurrent VTE (n=39)	N/A	Upregulated in recurrent VTE	(Wang et al., 2019)
Atrial fibrillation (AF)	plasma	acute new-onset AF (n = 5) well-controlled AF (n = 16) controls (n = 15)	↓	Downregulated in well-controlled AF compared to new-onset AF and control patients	(da Silva et al., 2018)
Hypertension (HT)	plasma	normotensive (n=15) hypertension (n=15)	↓	Significant correlation with systolic blood pressure	(Hijmans et al., 2018)
STEMI and SCAD	serum	STEMI (n=24) stable CAD (n=20)	N/A	Downregulated in STEMI	(Robinson et al., 2018)
Acute myocardial infarction (AMI)	plasma	post-AMI (n=44) controls (n=18)	↑	Early increase at day 4 compared to control patient levels, further increase up to day 9	(Grabmaier et al., 2017)

Ischemic stroke (IS) and Transient ischemic stroke (TIA)	serum	IS (n=177) TIA (n=81) controls (n=42)	↑	- Upregulated in IS vs. TIA and control patients - Higher in severe IS group vs. moderate IS and mild IS group	(Wu et al., 2017a)
Atrial fibrillation (AF)	serum	102 patients undergoing ablation for persistent AF	↑	- Correlation with extent of LVAs detected in the left atrium - Association with event-free survival after AF ablation	(Zhou et al., 2018a)
Lower extremity arterial occlusive disease (LEAOD)	plasma	LEAOD patients after interventional therapy (n=412)	N/A	- Higher in the restenosis group - Correlation with age, diabetes, and hypertension in the restenosis group	(Zhang et al., 2017a)
Acute coronary syndrome (ACS) and SCAD	serum	ACS (n=50) SCAD (n=50)	N/A	Higher in ACS vs. SCAD	(Darabi et al., 2016)
ACS	plasma	NSTE ACS (n=34) acute coronary syndromes without persistent ST-segment elevation (NSTE ACS)	N/A	- Inverse correlation with TC, LDL-C, and non-HDL-C - Independent predictor of non-HDL-C levels in patients with NSTE ACS with a less atherogenic lipid profile	(Miskowiec et al., 2016)
Congestive heart failure (CHF)	serum	HF (males, n=28)	N/A	Increase in response to an acute exhaustive exercise in CHF patients	(Xu et al., 2016)
Hypertrophic cardiomyopathy (HCM)	serum and plasma	HCM (n=28)	N/A	Upregulated in HCM patients with T1 < 470 ms vs. those with T1 ≥ 470 ms	(Fang et al., 2015)
Myocardial infarction (MI)	plasma	left ventricular remodeling (LVR) after MI (n=56) non-LVR after MI (n=142)	N/A	Higher in patients with LVR	(Liu et al., 2015)
Aortic stenosis and coronary artery disease	plasma	aortic stenosis (n=94) controls (n=101)	n.s.	- Increased in AS patients without CAD - No difference between groups with CAD	(Coffey et al., 2015)
Cardiac allograft vasculopathy (CAV)	plasma	CAD (n=80) CAV (n=52) healthy controls (n=25)	↑	- Increased in CAV vs. healthy controls - No increase in CAV-negative patients	(Singh et al., 2015)
Hypertension	plasma	HT (n=28) healthy controls (n=28)	↑	Positive correlation with systolic blood pressure, diastolic blood pressure, CRP and CIMT	(Cengiz et al., 2015)

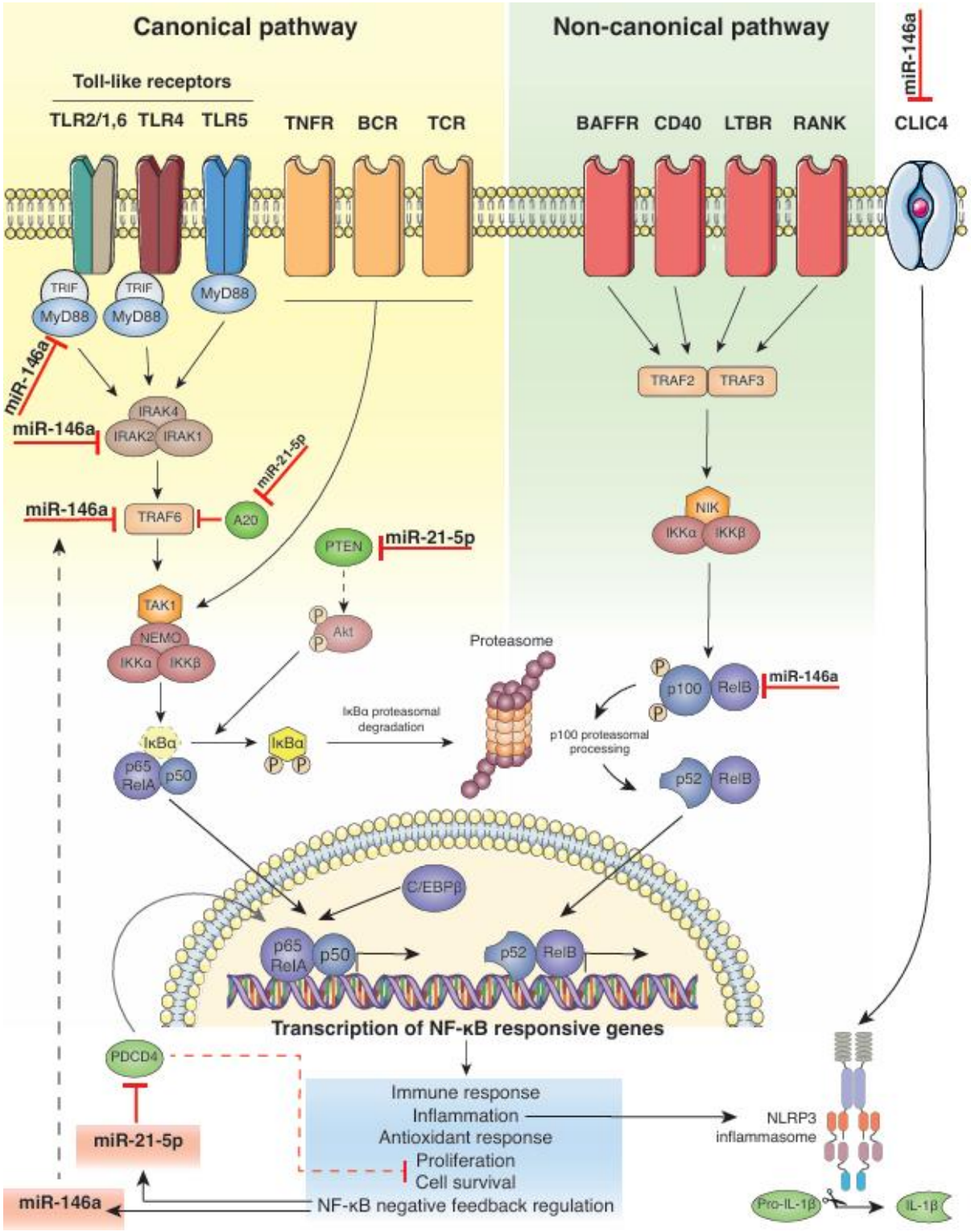
CAD	plasma	CAD (n=32) healthy controls (n=20)	↑		(Han et al., 2015)
Congestive heart failure (CHF)	serum	stable CHF (n=20) decompensated CHF (n=22) healthy controls (n=15)	↑	Upregulated in patients with systolic heart failure	(Cakmak et al., 2015)
Coronary atherosclerosis (CA)	plasma	EH (n=13) ARAS (n=13) CA (n=11) healthy volunteers (HV; n=13)	↑	Higher in CA vs. other groups	(Park et al., 2015)
Atrial fibrillation (AF)	plasma	AF (n=112) after AF ablation (n=47) no AF (n=99)	↓	Increasing levels after AF ablation	(McManus et al., 2015)
Acute myocardial infarction (AMI)	plasma	AMI (n=17) healthy volunteers (n=20)	↑	Correlation with plasma cardiac troponin I (cTnI)	(Wang et al., 2014)
Unstable angina (UA)	plasma	UA (n=45) stable angina (n=31) Controls (n=37)	↑	Upregulated in microparticles isolated from plasma of UA patients (n=5) vs. controls (n=5)	(Ren et al., 2013)
Nonalcoholic Fatty Liver Disease (NAFLD)	serum	NAFLD (males, n=48) NAFLD (females, n=44) no NAFLD (males, n=90) no NAFLD (females, n=221)	↑	Higher in men with NAFLD vs. men without NAFLD	(Yamada et al., 2013)
CVD	plasma	CVD (n=34) healthy controls (n=111)	↑	Correlation with CRP and fibrinogen	(Olivieri et al., 2012)
Acute coronary syndrome (ACS)	serum	ACS (n=106) no ACS (n=226)	↑	- Increased in patients diagnosed with ACS when initial troponin was still negative or with symptom onset <3 h - Adds diagnostic value to high-sensitivity troponin T in suspected ACS patients	(Oerlemans et al., 2012)
Aortic stenosis (AS)	plasma	surgical controls (n=32) AS (n=25) healthy controls (n=75)	↑	Higher in AS vs. controls (surgical and healthy control)	(Villar et al., 2013)
NSTEMI	plasma	NSTEMI (n=92) CHF (N=81) healthy controls (n=99)	↑	- Upregulated in NSTEMI vs. controls - Upregulated in STEMI vs. CHF	(Olivieri et al., 2013a)

Neurodegenerative diseases					
Late onset myasthenia gravis (LOMG)	serum	generalized LOMG (n=50) ocular LOMG (n=23) all LOMG (n=73)	N/A	- Decreased with the improvement of MG - Lower in ocular vs. generalized late onset MG	(Sabre et al., 2018)
Alzheimer's disease (AD) and dementia with Lewy bodies (DLB)	plasma extracellular vesicles (EVs)	DLB patients (n = 18) AD patients (n = 10) healthy individuals (n = 15)	↓ (AD)	- Downregulated in AD vs. controls - Downregulated in AD vs. DLB	(Gamez-Valero et al., 2019)
Alzheimer's disease (AD)	plasma	AD (n=116) and healthy controls (n=40) ≥ 65 yrs	=	- Higher in AD patients compared to healthy controls - Significant positive correlation between CRP and miR-21 in AD patients	(Giuliani et al., 2021)
Alzheimer's and Parkinson's diseases	serum EVs	AD (n=53) PD (n=50) control subjects (n=62)	↓ (AD)	- Downregulated in AD vs. controls - Upregulated in PD vs. AD	(Burgos et al., 2014)
Parkinson's disease	plasma	newly diagnosed and treatment-naïve PD (n=25) healthy controls (n=25)	↓		(Chen et al., 2018)
Alzheimer's disease and other neurodegenerative disorders	CSF	AD (n=10) Vascular dementia (n=4) frontotemporal dementia (n=2) dementia with Lewy bodies (n=2)	N/A	Downregulated in AD vs. other neurodegenerative disorders	(Sorensen et al., 2016)
Age-related macular degeneration (AMD)	serum	AMD (150 wet form, n=150 and dry form, n=150) controls (n=200)	↑	No difference between AMD forms	(Szemraj et al., 2015)
Parkinson's disease	blood	PD (n=15) controls (n=15)	↑		(Fu et al., 2017)
Age-related macular degeneration (AMD)	plasma	AMD (n=33) controls (n=31)	↑		(Ertekin et al., 2014)

FIGURE LEGEND

Figure 1. MiR-146a and miR-21 molecular targets in canonical and non-canonical pathways of NF-κB activation. In the canonical pathway, NF-κB activation is induced through the IKK (α and β subunits)-mediated phosphorylation of the inhibitory molecule IκB (IκBα, IκBβ and IκBγ), which in non-stimulated cells retains the NF-κB dimers in the cytoplasm. IKK-mediated phosphorylation of IκBα and the subsequent ubiquitin-dependent degradation by the 26S proteosome complex is needed to allow translocation of the hetero-dimeric p65(RelA)-p50 (derived from NF-κB1-p105) complex into the nucleus, driving the expression of responsive pro-inflammatory genes (Karin and Ben-Neriah, 2000) and miR-146a and miR-21-5p which act as endogenous negative feedback inhibitors. Notably, NF-κB1 (p105) is constitutively processed to p50 (Moorthy et al., 2006). In the non-canonical activation, NF-κB inducing kinase (NIK) activated by the TNF receptor-associated factor 3 (TRAF3) activates IKKα (two α subunits) which phosphorylates NF-κB2 (p100), so that it can be processed to the mature form p52. This allows for the assembly of the heterodimer RelB/p52(derived from NF-κB2) which can translocate into the nucleus to activate responsive genes (Scheidereit, 2006; Senftleben et al., 2001). Therefore, the activation of non canonical pathway is IκB-independent and requires active protein synthesis.

A20, tumor necrosis factor alpha-induced protein 3; Akt; protein kinase B; BAFFR, B-cell activating factor; BCR, B-cell receptor; C/EBPβ, CCAAT/enhancer-binding protein beta; CLIC4, chloride intracellular channel protein 4; LTBR, lymphotoxin beta receptor; NIK, NF-κB inducing kinase; NLRP3, NLR family pyrin domain containing 3; PDCD4, programmed cell death protein 4; PTEN phosphatase and tensin homolog; RANK, receptor activator of NF-κB; TCR, T-cell receptor; TLR, toll-like receptor; TNFR, tumor necrosis factor receptor; TRIF, TIR-domain-containing adapter-inducing interferon-β.


Canonical pathway
Non-canonical pathway
miR-146a
Toll-like receptors
TLR2/1,6 TLR4 TLR5
TNFR BCR TCR
BAFFR CD40 LTBR RANK
CLIC4
TRIF
MyD88
TRIF
MyD88
MyD88
miR-146a
IRAK4
IRAK2 IRAK1
miR-146a
miR-21-5p
miR-146a
TRAF6
A20
TRAF2 TRAF3
NIK
IKKα IKKβ
PTEN
miR-21-5p
TAK1
NEMO
IKKα IKKβ
P
P
Akt
Proteasome
P
P
p100 RelB
miR-146a
IκBα proteasomal degradation
p100 proteasomal processing
IκBα
p65 RelA p50
IκBα
P P
p52 RelB
C/EBPβ
p65 RelA p50
p52 RelB
Transcription of NF-κB responsive genes
PDCD4
miR-21-5p
Immune response
Inflammation
Antioxidant response
Proliferation
Cell survival
NF-κB negative feedback regulation
miR-146a
NLRP3 inflammasome
Pro-IL-1β
IL-1β