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Vascular ageing and endothelial cell senescence: Molecular mechanisms of physiology and diseases

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ABSTRACT

Ageing leads to a progressive deterioration of structure and function of all organs over the time. During this process endothelial cells undergo senescence and manifest significant changes in their properties, resulting in impairment of the vascular functionality and neo-angiogenic capability. This ageing-dependent impairment of endothelial functions is considered a key factor contributing to vascular dysfunctions, which is responsible of several age-related diseases of the vascular system and other organs. Several mechanisms have been described to control ageing-related endothelial cell senescence including microRNAs, mitochondrial dysfunction and micro environmental stressors, such as hypoxia. In this review, we attempt to summarize the recent literature in the field, discussing the major mechanisms involved in endothelial cell senescence. We also underline key molecular aspects of ageing-associated vascular dysfunction in the attempt to highlight potential innovative therapeutic targets to delay the onset of age-related diseases.

1. Introduction

The process of ageing implies a progressive accumulation of senescent cells across the tissues of all the body organs. Vascular ageing is associated with functional and structural alterations of endothelium, vascular smooth muscle cells and the extracellular matrix of blood vessels. The age-related decline of endothelial function becomes manifest through a reduced neo-angiogenic capacity and altered response to microenvironment stimuli such as reduced oxygen and nutrient supply. Vascular and microvascular alterations directly impact on ageing-associated organ damage (Gates et al., 2009; Iantorno et al., 2014). Circulation provides the interface for tissue delivery of nutrients and oxygen, transvascular exchange, removal of carbon dioxide and catabolic products and fluid body economy. Therefore, cell survival and organ functionality depend on adequate blood perfusion. Alteration of vasculature functionality occurs in physiological and premature ageing, thus affecting the general health, structure and functionality of all the body.

In this review we focus the attention on the molecular and pathophysiological mechanisms underlying age-related endothelial cells senescence, we also would like to underline the importance of understanding the mechanisms at the basis of these processes in order to design targeted therapy to prevent organ damage in physiological and premature ageing.

Abbreviations: VEGF, vascular endothelial growth factor; miR, microRNA; HUVEC, human vein endothelial cells; BECN1, Beclin1; HIFs, hypoxia inducible factors; PDGFb, platelet-derived growth factor B.

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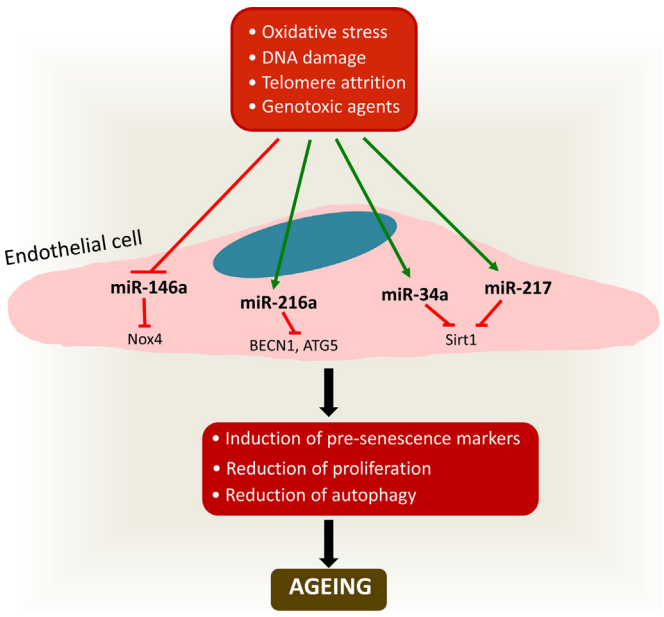

Fig. 1. MicroRNAs are involved in different biological processes of endothelial cell biology and that can also determine endothelial dysfunction. They are induced by different events occurring during ageing including oxidative stress, DNA damage, telomere attrition, and genotoxic agents. Over-expression of senescence-associated miRNAs might include alteration of proliferation potential, autophagy and senescence that can eventually culminate in vascular ageing. MicroRNAs involved in endothelial cell ageing are: miR-34a and miR-217, targeting sirt1; miR-216a targeting BECN1 and ATG5 and miR-146a targeting NOX4. See text for further explanations.

1.1. MicroRNAs in ageing-induced endothelial cell senescence

MicroRNAs (miRNAs/miRs) have been crucially involved in several diseases (Boon and Dimmeler, 2015; Candi et al., 2015; Santulli, 2015; Su et al., 2015), including vascular pathologies clearly associated with increasing ageing. MiRNAs are small non-coding RNAs acting at post-transcriptional level on target mRNAs to repress gene expression. By binding complementary sequences at the 3′-end of mRNAs, miRNAs cause mRNA degradation and/or translation inhibition. Several miRNAs and miRNA clusters including miR-146a, miR-34a, miR216 and miR217 (Liu et al., 2014; Meng and Kaufmann, 2014; Menghini et al., 2009; Menghini et al., 2014; Vasa-Nicotera et al., 2011), have been functionally linked to endothelial cell senescence (Fig. 1).

MiR-146a expression is reduced in response to senescence induction by serial passaging in human vein endothelial cells (HUVEC). Overexpression of miR-146a in senescent endothelial cells reduced the number of senescence-associated (SA)-beta galactosidase positive cells, suggesting its anti-senescence action. Expression of miR-146a in cells significantly inhibited NOX4 protein expression. NOX4 is the predominant NOX isoform in endothelial cells; its down-regulation results in NOX4-reactive oxygen species (ROS) reduction (Vasa-Nicotera et al., 2011; Yan et al., 2014) in cells.

Endothelial senescence is also controlled by miR-217 and miR-34a as depicted in *in vitro* studies (Menghini et al., 2009). Overexpression of miR-217 in HUVEC resulted to an early senescent-like phenotype. Conversely, miR-217 knock-down in senescent cells delayed/reduced senescence markers. MiR-217 targets Sirtuin-1 (Sirt1) mRNAs, resulting in reduced protein expression (Menghini et al., 2009). Sirt1 is a class III histone deacetylase involved in deacetylation of many proteins, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and peroxisome proliferator-activated receptor gamma (PPRG), and its expression is progressively reduced in multiple tissues during ageing. Several studies have suggested that Sirt1 plays a protective role in endothelium and its knock-down has been shown to induce an early senescent status in endothelial cells, paralleled by a reduction in endothelial nitric oxide synthase (eNOS) and forkhead box protein O1 (FoxO1), both of which are important factors for endothelial cells proliferation and angiogenesis (Liu et al., 2015; Zhang et al., 2007; Zhou et al., 2014). Sirt1 is also targeted by miR-34a to trigger endothelial cell senescence. Forced expression of Sirt1, abrogated the effect of miR-34a on endothelial senescence. Interestingly, Sirt1 down-regulation resulted in p53 acetylation and activation, which in turn causes miR-34a expression, resulting in a positive feedback loop that accelerates Sirt1 suppression (Menghini et al., 2009). Interestingly, miR-34a affects also endothelial progenitor cells, necessary to replace endothelium with new healthy cells. Indeed, overexpression of miR-34aimpaired endothelial progenitor cells angiogenesis *in vitro* (Zhao et al., 2010).

It has been shown that the age-associated reduction of autophagic activity may play a causative role in the development of several diseases, including vascular pathologies (Agostini et al., 2011; Amelio et al., 2011; Kim et al., 2013; Lavandero et al., 2015; Li et al., 2014; Nussenzweig et al., 2015; Vindis, 2015). MiR-216a is induced during endothelial ageing and directly targets two autophagy related genes, Beclin1 (BECN1) and ATG5 (Lin et al., 2014; Ma et al., 2014; Menghini et al., 2014). The inverse correlation between miR216a and BECN1 and ATG5 has been validated *in vivo* in human atherosclerotic plaques and in myocardial biopsies of patients with heart failure, indicating the importance of autophagy in endothelial dysfunction and heart disease (Menghini et al., 2014).

Taken together, these results implicate functional roles for several miRNAs, such as miR-146a, miR-217, and miR-34a, in endothelial cell senescence (Fig. 1) both as positive and negative regulators. In particular, miR34a, miR-216a and miR217 are induced in senescent cells, by different stimuli. By modulating selected targets (sirt1, BECN1 and ATG5) they reduce proliferation and autophagy, therefore facilitating ageing. On the other hand, miR146a is down-regulated in senescent cells, resulting in increased NOX4 and reduction in ROS levels. Further studies will be required to determine if these miRNAs dynamically regulate vascular pathologies and their consequences in elderly subjects.

Finally, MiR-92a is also highly expressed in endothelial cells but its inhibition increases angiogenesis *in vitro* as well as *in vivo*. Indeed, systemic inhibition of miR-92a results in an increased recovery in a mouse model of hind limb ischemia as well as recovery after acute myocardial infarction (Bonauer et al., 2009), indicating a possible miR-92a function in counteracting endothelial cells senescence; however the mechanism at cellular/molecular level, has not been investigated yet.

1.2. Transglutaminase 2 in angiogenesis and age-related vascular stiffness

Limiting angiogenesis affects various clinical contexts, including ageing. Proteins of the vascular endothelial growth factor (VEGF) family are key angiogenic factors. Selected VEGF isoforms bind the receptor VEGFR2 and heparan sulfate proteoglycans to stimulate angiogenesis, recently it has been shown that transglutaminase 2 (TG2) enzyme play a role in VEGF165-heparan sulfate proteoglycans interaction (Beckouche et al., 2015). TG2 is a Ca2+-dependent cross-linking enzyme that catalyzes the formation of ε-γ-glutamyl isopeptide bonds between peptide-bounds glutamine and lysine (Candi et al., 2005; De Laurenzi and Melino, 2001; Liu et al., 2002; Myneni et al., 2015; Rossin et al., 2015; Shrestha et al., 2015). TG2 is highly expressed in endothelial cells, it is located in the cytoplasm and in extracellular space. In hypoxic condi-

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