Alternative Splicing in Endothelial Senescence: Role of the TGF-β Co-Receptor Endoglin

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1. Introduction

The vascular endothelium is the thin monolayer of specialized cells that line the blood vessels of the cardiovascular system. This endothelium is more than a simple protective barrier since it possesses anticoagulatory properties, mediates the metabolites exchange and regulates the vascular tone and homeostasis maintenance. These functions are finely tuned by endothelial cells that, in the absence of any stimuli, remain in a quiescent stage (Conway & Carmeliet, 2004). In fact, endothelial cells occasionally divide in a normal vessel, displaying a very low turnover rate except for localized areas (Foteinos et al., 2008). Thus, the endothelium is quite sensitive to a variety of signals including shear stress and circulating factors that lead to endothelial activation. As a result of their own physiology along the lifespan, endothelial cells progressively accumulate reactive oxygen species and pro-oxidant metabolites due to an increased oxidative stress, damages in DNA and advanced cellular replication involving shortening of telomeres. Altogether, these alterations lead endothelial cells to reach senescence (Brandes et al., 2005; Foreman & Tang, 2003), which has been proposed to be at the cellular basis of most of the vascular pathologies associated with ageing, such as atherosclerosis or hypertension (Minamino & Komuro, 2008; Rodríguez-Mañas et al., 2009).

The major aspect of endothelial physiology implies the growth or formation of new blood vessels from pre-existing ones, process named angiogenesis which is mainly induced by metabolic requests (Fraisl et al., 2009). Angiogenesis plays a key role from the first steps during the embryonic development to the adult stage, and is involved in numerous physiological processes such as wound repair or the growth of the tissues (Carmeliet & Jain, 2011). However, angiogenesis and vascular remodelling decline with age and several lines of evidence indicate that ageing and endothelial dysfunction progress in parallel (Brandes et al., 2005; Ferrari et al., 2003; Minamino et al., 2004). In this sense, numerous efforts are addressed to elucidate the molecular mechanisms that underlie vascular ageing.

2. TGF-β in angiogenesis - Role of endoglin

The angiogenesis process consists of two separate but balanced phases, activation and resolution, that are finely arranged by a suite of cytokines, among which the transforming

growth factor (TGF)-β plays a dual role (Pardali et al., 2010). TGF-β is the prototypic member of a large family of multifunctional and evolutionarily conserved cytokines, including also activins and bone morphogenetic proteins (BMPs). Upon proteolytic activation, TGF-β circulates as a 25 kDa homodimer that elicits its cellular functions by binding to a membrane complex of type II (TβRII) and type I (TβRI or ALKs) receptors with cytoplasmic serine-threonine kinase activity (Kang et al., 2009). Endothelial cells express two different TβRIs, named ALK5 and ALK1, with distinct affinity for the ligand and different signalling pathways mediated mainly by Smad proteins (Smad2/3 and Smad1/5/8, respectively) (Massague et al., 2005). Moreover, endothelial cells also express endoglin, or CD105, an auxiliary TGF-β receptor that modulates the balance between ALK1 and ALK5 signalling. Endoglin is mainly expressed as a homodimeric protein of 180 kDa and is associated to the activation phase of angiogenesis, acting as a modulator between both phases. In this context, endoglin interacts with ALK1 and promotes the TGF-β/ALK1 signalling pathway (Blanco et al., 2005; Lebrin et al., 2004).

The TGF-β/endoglin pairing has been studied in different contexts such as differentiation (Tang et al., 2011), cancer (Bernabeu et al., 2009; Perez-Gomez et al., 2010) and other pathologies including liver fibrosis (Meurer et al., 2011) or preeclampsia (Venkatesha et al., 2006). However, endoglin plays a major role in angiogenesis as well as in vascular remodelling and homeostasis (Lopez-Novoa & Bernabeu, 2010; ten Dijke et al., 2008). Heterozygous mutations in the endoglin gene (*ENG*) are responsible for the vascular dysplasia named hereditary haemorrhagic telangiectasia (HHT) type 1 (McAllister et al., 1994; Shovlin, 2010), a rare genetic disease with autosomal dominant inheritance. These mutations lead to the development of abnormal vascular structures that are the basis of the characteristic HHT symptoms, including frequent and recurrent nosebleeds, telangiectases in the nasal and gastrointestinal tracts and large arteriovenous malformations in different organs such as lung, liver or brain (Mahmoud et al., 2010; Shovlin, 2010). Nonetheless, the HHT symptoms are not present at birth and normally appear during adolescence, getting worse with age. This is in line with the functional role of endoglin in angiogenesis and with previous observation that angiogenesis becomes impaired with ageing (Rivard et al., 1999).

2.1 Two alternatively spliced endoglin isoforms

Most of published studies about endoglin are referred to L-endoglin (long endoglin) that is the predominantly expressed isoform. However, the expression of a short variant (S-endoglin) was described first in humans (Bellon et al., 1993) and later in mouse (Perez-Gomez et al., 2005). In humans, both isoforms share the identical large extracellular region and the transmembrane domain, so that the only difference resides in their cytoplasmic tails (Figure 1A). In the case of L-endoglin, this region is composed by 47 amino acids with a high frequency of serine and threonine residues susceptible to be phosphorylated. Also, the sequence serine-methionine-alanine, SMA, in the C-terminal end is a docking site for proteins with a PDZ domain and is involved in the cytoskeleton organization (Koleva et al., 2006). By contrast, the sequence of the S-endoglin cytoplasmic tail is 14 amino acids long and contains only one serine and threonine residues; also the last 7 residues are specific for this isoform (Figure 1B). These data suggest that L-endoglin and S-endoglin may elicit different functional effects on the endothelial cell.
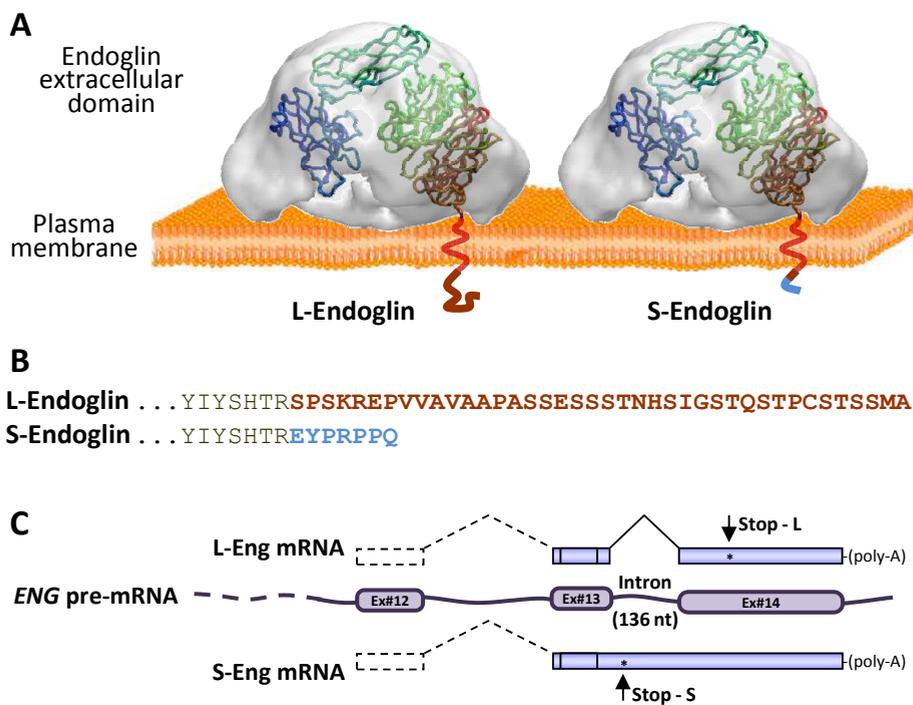
Fig. 1. The two endoglin isoforms. (A) The electron microscopy density map (grey) of the endoglin extracellular region shows the overall structure. The backbone of a theoretical atomic model of the endoglin monomer is fitted inside (adapted from Llorca et al., 2007). This structure is common to both endoglin variants. The transmembrane domain (red) and cytoplasmic tails (brown, L; blue, S) are schematized. (B) The amino acid sequence of the cytoplasmic domain is detailed for both isoforms. (C) The endoglin pre-mRNA is represented in the middle of the mature transcripts that originate each isoform. The retention of the final intron by an alternative splicing process leads to S-endoglin expression.

S-endoglin arises as the result of an alternative splicing mechanism by which the last intron, between exons #13 and #14, is retained in the mature mRNA (Figure 1C). Consequently, an early stop codon appears in the open reading frame and truncates the mature protein in the cytoplasmic region. Although this mechanism of intron retention normally involves a rapid degradation by the nonsense-mediated decay machinery (Lareau et al., 2004; Nott et al., 2003), under certain conditions it may also lead to a biologically active isoform (Sakabe & de Souza, 2007); and this is the case of endoglin. Thus, when endothelial cells become senescent during the ageing process, they show an up-regulation of S-endoglin (Blanco et al., 2008). At this senescent stage, both endoglin isoforms are co-expressed likely forming heterodimers, as it occurs in mice (Perez-Gomez et al., 2005), and some of the cellular responses to TGF-β are oppositely regulated by each isoform. Indeed, the S-endoglin increase has an antiangiogenic role in the blood vessels and contributes to vascular pathology (Blanco et al., 2008; Perez-Gomez et al., 2005; Velasco et al., 2008).

3. Endothelial senescence and TGF-β

It is well known that ageing *per se* is the major risk factor for the development of cardiovascular diseases. Thus, senescence has been widely and mainly analyzed in *in vitro* studies but there are also evidences that this process takes place *in vivo* (Erusalimsky & Kurz, 2005; Minamino & Komuro, 2007). The first evidence of cellular senescence in primary cultures *in vitro* is the deceleration in the proliferation, that is, an increase in the doubling time of the cell population. In parallel, cells experience morphological changes along theses passages that involve the augment of the cellular size and shape. However, these observations are usually complemented with a useful tool based on the abnormal behaviour associated with senescent cells of the lysosomal hydrolase β-galactosidase. Thus, the senescence-associated β-galactosidase (SA β-gal) activity at pH 6 is widely accepted as an easily detectable senescence histochemical marker (Dimri et al., 1995).

Endothelial senescence is a cellular process that is clearly linked to both ageing and the development of vascular pathologies as well (Brandes et al., 2005; Erusalimsky, 2009; Minamino & Komuro, 2007). Basically, senescence constitutes a stress and damage response phenomenon that involves a permanent growth arrest (Campisi & d'Adda di Fagagna, 2007). Consequently, senescent cells undergo diverse changes in gene and protein expression that lead to an impairment of cellular functions (Foreman & Tang, 2003; Young & Narita, 2009). Thus, these changes usually affect to the endothelial phenotype favouring a pro-inflammatory, pro-atherosclerotic, or a prothrombotic state (Erusalimsky, 2009).

Here, TGF-β plays an important role owing to its ability to prompt senescence in a variety of cell types (Cipriano et al., 2011; Kordon et al., 1995; Tremain et al., 2000; van der Kraan et al., 2011; Wu et al., 2009). In the vascular context, it has been reported, e. g., elevated levels of TGF-β in the aging varicose veins that likely favour the fibrous process and the consequent venous insufficiency (Pascual et al., 2007). In this sense, the profibrotic effect of TGF-β is mediated by the stimulation via Smad3 signalling of the plasminogen activator inhibitor (PAI)-1 expression, a key regulator of the synthesis and deposition of the extracellular matrix in the tissue homeostasis (Ghosh & Vaughan, 2011). Thus, the increase of TGF-β up-regulates PAI-1 expression, which contributes to the accumulation of collagen and other extracellular matrix components. This PAI-1 increase is also in line with the decrease of the antithrombogenic properties of a senescent endothelium due to the inhibition of the urokinase- and tissue-type plasminogen activator (uPA and tPA, respectively)/plasmin axis (Comi et al., 1995; Schneiderman et al., 1992).

3.1 Replicative senescence

Senescence was initially considered to reflect the finite capacity for division that normal diploid cells exhibit when propagated in culture. This statement is based on the successive rounds of cell division that imply the progressively shortening and eventual dysfunction of telomeres, the physical ends of chromosomes, in a phenomenon known as Hayflick's limit (Hayflick, 2003; Shay & Wright, 2007). Thus, the down-regulation of telomerase, the enzyme responsible for maintaining the telomeres length, is clue for the senescence program. Besides, because telomerase is re-activated in the majority of neoplastic processes, it is postulated that inhibiting telomerase activity should result in senescence induction by telomere shortening which can cause the death of cancer cells (Folini et al., 2011). Interestingly, the senescence inducer TGF-β down-regulates the telomerase activity. Thus,

upon TGF-β treatment, Smad3 is able to interact with the transcription factor c-myc, so repressing the promoter of the hTERT gene, encoding the catalytic subunit of telomerase (Figure 2). Thus, the c-myc activity is blocked in the Smad3 complexes which negatively affects to the cell cycle (Li & Liu, 2007; Li et al., 2006). In addition, this repression of the hTERT promoter mediated by TGF-β can be alternatively reinforced by the activation of the TGF-β activated kinase (TAK)-1 pathway that abrogates the transcriptional activity of Sp1 on the hTERT promoter (Fujiki et al., 2007).
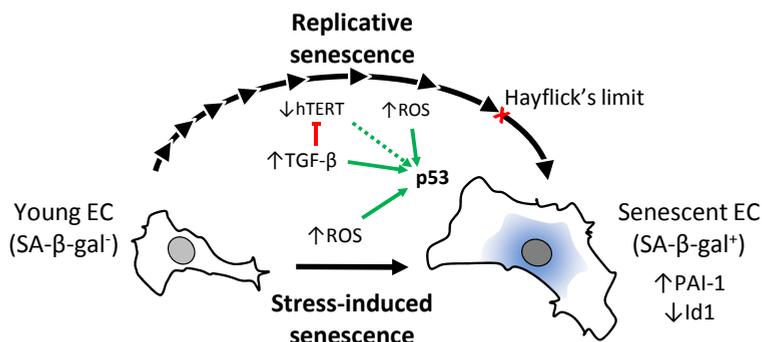

Fig. 2. The endothelial senescence. Endothelial cells extensively cultured *in vitro* enlarge their size and shape, showing a positive blue staining for the SA-β-gal activity. Endothelial senescence is reached by, at least, two different routes, including replicative or oxidative stress-induced. Both pathways involve the activation of p53 and are characterized by an increase in PAI-1 expression and the repression of Id1.

The characteristic and irreversible growth arrest observed in senescent cells occurs in the transition from phase G1 to phase S of the cell cycle and is dependent on the retinoblastoma family proteins, playing the tumour suppressor p53 a key role which senses the telomeric DNA damage (Wesierska-Gadek et al., 2005). In this transition, the abolition of p53 expression interferes with the senescence process that would be related to the low levels of PAI-1, one of the p53 target genes (Kortlever et al., 2008). Conversely, it is well known that the p53 overexpression or activation is able to arrest the cell cycle and launch the senescence program, suggesting that this process could be useful in cancer therapy (Chen & Goligorsky, 2006; Ewald et al., 2010; Rosso et al., 2006; Sugrue et al., 1997). Furthermore, it was demonstrated that the prolonged treatment with interferon (IFN)-γ induces cellular senescence in endothelial cells, involving cell cycle arrest and an up-regulation of p53 and p21 proteins cells (Kim et al., 2009).

Another TGF-β target protein that is associated with endothelial senescence is the helix-loop-helix (HLH) transcription factor Id1, or inhibitor of DNA binding 1. Id1 lacks a basic DNA-binding domain, but is able to form heterodimers with other HLH proteins, thereby inhibiting DNA binding, a process that is essential for cellular proliferation (Benezra et al., 1990). In epithelial cells, TGF-β induces the formation of a Smad3/ATF3 heteromeric complex that represses the Id1 expression and negatively regulates the cell cycle (Kang et al., 2003). Hence, the decrease in the Id1 expression is considered a biomarker of endothelial senescent cells (Tang et al., 2002).

3.2 Oxidative stress-induced senescence

Endothelial senescence can also be triggered by telomere-independent events that in general involve damages in the DNA. In this sense, the oxidative stress is a major stimulus for the induction of this type of senescence, which is due to the generation of reactive oxygen species (ROS, including oxygen ions and peroxides) in the mitochondria (Collins & Tzima, 2011; Erusalimsky & Skene, 2009). Thus, the cellular metabolism is the central source of ROS, but often they have an extracellular origin such as the one induced by radiation. In any case, ROS can either provoke or accelerate the development of senescence by damaging the DNA (Figure 2), which triggers multiple response mechanisms that usually act through the retinoblastoma protein family pathways, the final effectors of the senescence program (Campisi & d'Adda di Fagagna, 2007; Erusalimsky, 2009).

In cell culture, ROS induce an acute form of senescence termed stress-induced premature senescence, which does not require extensive cell culture but which resembles somehow the replicative one (Toussaint et al., 2000). This type of senescence is relatively easy to analyze in *in vitro* assays because the sole treatment with hydrogen peroxide (H_2O_2) for a short lapse of time is enough to prompt this type of senescence (Chen et al., 1998). By contrast, using antioxidant agents such as the grape stilbenoid resveratrol protect from the oxidative stress-induced premature senescence (Kao et al., 2010). Also, several lines of evidence show that ROS can interact and deplete the nitric oxide (NO) generated by the endothelium in the vasodilator responses, so contributing to the endothelial dysfunction associated to ageing (Grisham et al., 1998; Steiner et al., 2002). This is in line with the availability of NO-donors to inhibit endothelial cell senescence (Hayashi et al., 2006). In fact, comparing elderly with young adults one can find that the NO levels, or its bioavailability, are decreased in the first group but, interestingly, without any difference regarding to the expression levels or activation state of the endothelial nitric oxide synthase (eNOS), the enzyme responsible of the NO generation (Sun et al., 2004; Taddei et al., 2001). In parallel, this decrease in the NO levels attenuates the negative interference that it exerts on the TGF-β signalling pathway (Saura et al., 2005), which contributes to prompt the senescence program.

On the other hand, radiation is an exogenous trigger for ROS. In human skin fibroblasts, repeated exposure to ultraviolet-B light at subcytotoxic level is able to prompt premature senescence. Interestingly, this effect is mediated by the increase in the TGF-β expression and consequently by its downstream signalling pathway (Debacq-Chainiaux et al., 2005). In the vascular context, this source of ROS has been poorly studied beyond the methodological interest to induce premature senescence because endothelial cells enter rapidly in apoptosis due to their high sensitivity to radiation (Paris et al., 2001). In this regard, a recent study has demonstrated that ionizing radiation suppresses angiogenesis in mice and this effect is mediated through the TGF-β/ALK5-dependent inhibition of endothelial cell sprouting (Imaizumi et al., 2010).

4. Induction of S-endoglin and its role in endothelial senescence

The molecular changes involved or associated to the senescent program not only concern to the induction or repression of a specific set of genes. Many of the changes described in the literature report post-translational modifications, e. g., the advanced glycation endproducts

(AGEs) which have been implicated in age-related disease and aging itself; as well as the p53 acetylation in stress-induced senescence (Furukawa et al., 2007). In addition, a growing body of evidence supports the involvement of the post-transcriptional modifications that occur in senescence, i. e., the alternative splicing processes associated with senescence (Harries et al., 2011; Meshorer & Soreq, 2002). Thus, alterations in the splicing pattern have been described for several age-related diseases, such as the Hutchison Gilford progeria syndrome (Eriksson et al., 2003), or the Alzheimer's disease-related tauopathies (Chen et al., 2010).
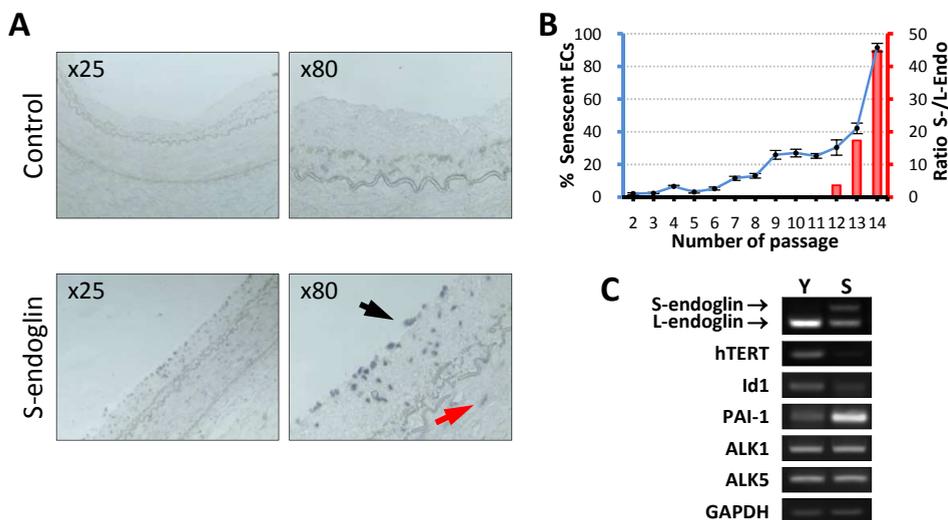

Fig. 3. S-endoglin expression in senescence. (A) The expression of S-endoglin in blood vessels can be revealed by *in situ* hybridization in the endothelium of human coronary artery (black arrow) and in some smooth muscle cells (red arrow). (B) The increase in the percentage of senescent endothelial cells *in vitro* (blue graph) is concomitant with the induction of S-endoglin (red graph). (C) Primary cultures of human umbilical vein endothelial cells (HUVECs) maintained *in vitro* along passages co-express both endoglin isoforms comparing young (Y) versus senescent (S) cells in RT-PCR assays. In parallel, PAI-1 is increased, while Id1 and telomerase (hTERT) are down-regulated in senescent cell. As a control, the expression levels of the TGF-β type I receptors ALK1 and ALK5 are not altered. (Figure adapted from Blanco et al., 2008).

Nonetheless, little is known about the role of splicing in the vascular context during senescence. A recent study demonstrates that TGF-β induces the distal splice-site selection leading to an antiangiogenic variant of the vascular endothelial growth factor (VEGF) (Nowak et al., 2008), and this could be one of the reasons why there is a reduced capability to form tubular-like structure by senescent endothelial *in vitro* (Chang et al., 2005).

As described above, the role of TGF-β in senescence has been clearly established, modulating specific intracellular effectors and leading to the cell growth arrest. In a first

step, TGF-β binds to the specific receptor complex at the endothelial cell surface. Then, the signal is transmitted into the cytoplasm by different pathways depending on the type I receptor present in the complex. Thus, ALK5 signals via Smad2 and Smad3, whereas ALK1 mainly activates Smad1 and Smad5. In the TGF-β receptor complex, the presence of the predominantly expressed isoform, L-endoglin, favours the ALK1/Smad1 pathway and is related to the activation phase of the angiogenesis (Blanco et al., 2005; Lebrin et al., 2004). However, a post-transcriptional change during endothelial senescence, such as the retention of the last and small intron in the endoglin mRNA, has important consequences. Thus, the up-regulation of S-endoglin *in vitro* and *in vivo* is clearly associated with the ageing (Figures 3A and 3B). The co-expression of S- and L-endoglin in the senescent endothelial cells is able to tilt the angiogenic balance toward the resolution phase (ALK5/Smad3 pathway) in detriment of the ALK1/Smad1 route (Blanco et al., 2008). Also, S-endoglin induces the up-regulation of the PAI-1 and the repression of Id1, changes clearly associated to the cell cycle arrest in senescence (Figure 3C and 4).
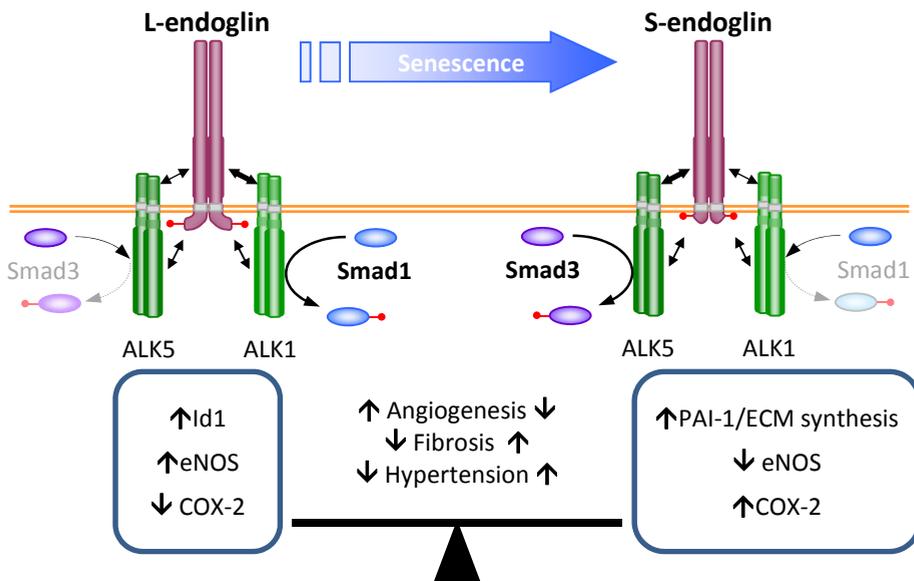

Fig. 4. Functional effects of S-endoglin in endothelial senescence. The S-endoglin up-regulation in aged endothelial cells promotes the ALK5/Smad3 signalling pathway. As a consequent, the vascular physiology is affected decreasing the angiogenesis, increasing the fibrosis and unbalancing the eNOS/COX-2 system which is related to hypertension. (Figure adapted from Blanco et al., 2008)

Furthermore, transgenic mice that overexpress the human S-endoglin isoform (*S-Eng*[+]) experience a significant increase in the mean arterial pressure and a failure in the control on the NO-dependent vascular homeostasis, similarly to what happens in the endoglin deficient mouse model (*Eng*^{+/-}) that resembles the HHT disease (Blanco et al., 2008; Santibanez et al., 2007). Supporting this, a common compensatory mechanism takes place in *S-Eng*[+] and *Eng*^{+/-} mice involving the up-regulation of the cyclooxigenase (COX)-2 enzyme

(Blanco et al., 2008; Jerkic et al., 2006). Taken together, the induction of S-endoglin during endothelial senescence might be at the basis of the development of cardiovascular pathologies associated with ageing, including atherosclerosis and hypertension (Figure 4).

4.1 Regulation of endoglin alternative splicing in senescence

Briefly, the alternative splicing is a molecular process by which organisms notably increase the diversity and functionality of their proteome from a finite number of genes. This process is carried out by the spliceosome, a huge ribonucleoprotein complex that works with amazing fidelity: i) skipping or shuffling exons; ii) selecting alternative splice sites; or iii) retaining introns (Graveley, 2001; Kwan et al., 2007). In humans, there are two distinct spliceosome complexes, named the major (M-Sp) and the minor (m-Sp) spliceosome. The M-Sp is involved in the vast majority of the splicing events and comprises five snRNPs named U1, U2, U4, U5, and U6 and a multitude of non-snRNP splicing factors (Jurica & Moore, 2003; Matlin et al., 2005; Zhou et al., 2002). Likewise, the m-Sp is composed by four unique snRNPs, U11, U12, U4atac, and U6atac, besides the U5 snRNP shared by both spliceosomes (Hall & Padgett, 1996; Tarn & Steitz, 1996). The m-Sp was first associated with the maturation of the so-called non-canonical introns but its role on standard splicing has been recently reported (Sheth et al., 2006; Will & Luhrmann, 2005). Interestingly, the difference between the major spliceosome and the minor spliceosome is their spatial segregation. While the M-Sp is in the nucleus, the m-Sp can be detected in the cytosol (Caceres & Misteli, 2007; Konig et al., 2007). In both cases, the spliceosome assembly is driven by a set of snRNPs that sequentially recognize the 5' and 3' splice sites, as well as the branch point element in between them (Burge et al., 1999). These snRNPs constitute the basal machinery of the spliceosome, besides a number of essential proteins that takes part in the spliceosome assembly. Moreover, there are several groups of auxiliary proteins that may regulate the alternative splicing. These splicing factors, or *trans*-elements, recognize binding sites, or *cis*-elements, spatially distributed inside the introns or exons and act as silencers or enhancers (Moore & Silver, 2008; Singh & Valcarcel, 2005; Sperling et al., 2008; Wang et al., 2006). Unfortunately, the alternative splicing during endothelial senescence has been poorly studied so far, but its importance has been suggested by the lifespan extension provoked by the overexpression of the splicing factor SNEV (Voglauer et al., 2006).

One of the best characterized groups of splicing factors is the serine/arginine (SR) protein family, from which the alternative splicing factor/splicing factor 2 (ASF/SF2) is the prototypical member (Graveley, 2000). ASF/SF2 is involved in both constitutive and alternative splicing processes. Although ASF/SF2 is mainly found in the nuclear speckles, it continuously shuttles between the nucleus and the cytoplasm depending on the phosphorylation and/or methylation states, which in turn determines its activity (Sanford et al., 2008; Sanford et al., 2005; Sinha et al., 2010). In this context, it has been recently reported the role of ASF/SF2 in the regulation of the S-endoglin intron retention during endothelial senescence (Blanco & Bernabeu, 2011). In endothelial senescent cells, the subcellular pattern of ASF/SF2 is mainly cytoplasmic, where ASF/SF2 interferes with the minor spliceosome inhibiting the elimination of the last intron of endoglin mRNA. The role of cytoplasmic ASF/SF2 as a senescent inductor is supported by its antiangiogenic properties, because the inhibition of the ASF/SF2 phosphorylation promotes its cytoplasmic localization and this is associated with increased expression levels of the antiangiogenic isoform VEGF165b (Nowak et al., 2010).
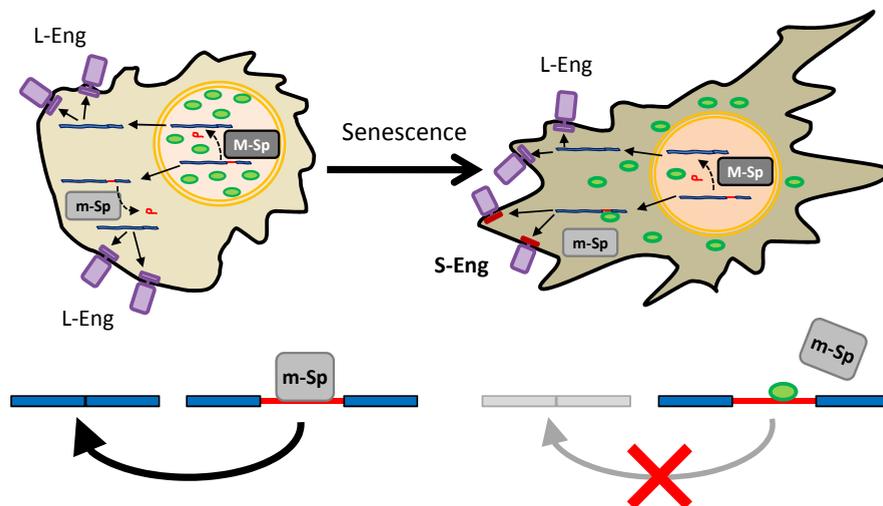
Fig. 5. Regulation of the alternative splicing of endoglin in senescent endothelial cells. In this hypothetical model, the last intron of the *ENG* gene is eliminated in the mature mRNA, so that L-endoglin is the predominantly expressed isoform. In this mRNA processing, both spliceosomes (nuclear M-Sp and cytoplasmic m-Sp) can be involved. However, in senescent endothelial cells, the splicing factor ASF/SF2 (green) is translocated to the cytoplasm, stabilizing the S-endoglin mRNA and interfering with the m-Sp activity. Consequently, ASF/SF2 promotes the intron retention, thus up-regulating the levels of S-endoglin mRNA (adapted from Blanco & Bernabeu, 2011).

5. Conclusions

Vascular physiology progressively declines with age due to multiple factors including an increase in oxidative stress, DNA damage, and advanced cellular replication involving telomere attrition. All these events converge in the key molecule p53, which acts typically arresting the cell cycle and triggering the endothelial senescence. At this stage, the expression of many specific genes is modulated, regarding not only to their expression levels but also the post-translational modifications and alternative processing of their premature mRNA molecules, which give rise to interesting protein variants. Nowadays, it can be postulated that this phenomenon is at the cellular basis of several age-associated cardiovascular pathologies, such as hypertension or atherosclerosis.

TGF-β is able to induce endothelial senescence via a cell surface receptor complex that includes the type I (ALK1 and ALK5) and the type II signalling receptors as well as endoglin. Endoglin is a TGF-β co-receptor highly expressed as L-(long)-endoglin by endothelial cells which is associated with active angiogenesis foci and vascular remodelling processes. Conversely, an alternative spliced and shorter isoform (S-endoglin) with opposite effects to those of L-endoglin in the context of the TGF-β system has been described. Usually, S-endoglin is almost undetectable in endothelial cells, but is induced during senescence. In this up-regulation, the senescence-induced cytoplasmic localization of the splicing factor ASF/SF2 plays a key role favouring the retention of the intron between exons

#13 and #14. Thus, the up-regulated expression of S-endoglin is considered to be part of the endothelial senescence program. Moreover, *in vitro* and *in vivo* studies suggest that S-endoglin contributes to vascular pathology associated with ageing. In this regard, mutations in the human *ENG* gene are responsible for HHT-1, an autosomic dominant vascular disease whose symptoms increase and become worse with age. Currently, the haploinsufficiency of the predominantly expressed L-endoglin isoform is widely accepted as the pathogenic mechanism of the disease. Because S-endoglin is up-regulated in aged mice as well as during senescence of endothelial cells and S-endoglin counteracts the function of L-endoglin, the increased S-endoglin expression during ageing would increase the functional L-endoglin haploinsufficiency in HHT-1 and could explain why the symptoms become worse with ageing. Therefore, one could predict that the age-dependent penetrance of the HHT-1 is due, at least in part, to the S-endoglin induction mediated by ASF/SF2.

In summary, these data suggest an important role for the TGF-β co-receptor endoglin as a modulator of the vascular pathology associated with endothelial senescence.

6. Acknowledgments

7. References

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Senescence

Edited by Dr. Tetsuji Nagata

The book "Senescence" is aimed to describe all the phenomena related to aging and senescence of all forms of life on Earth, i.e. plants, animals and the human beings. The book contains 36 carefully reviewed chapters written by different authors, aiming to describe the aging and senescent changes of living creatures, i.e. plants and animals.

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