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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.

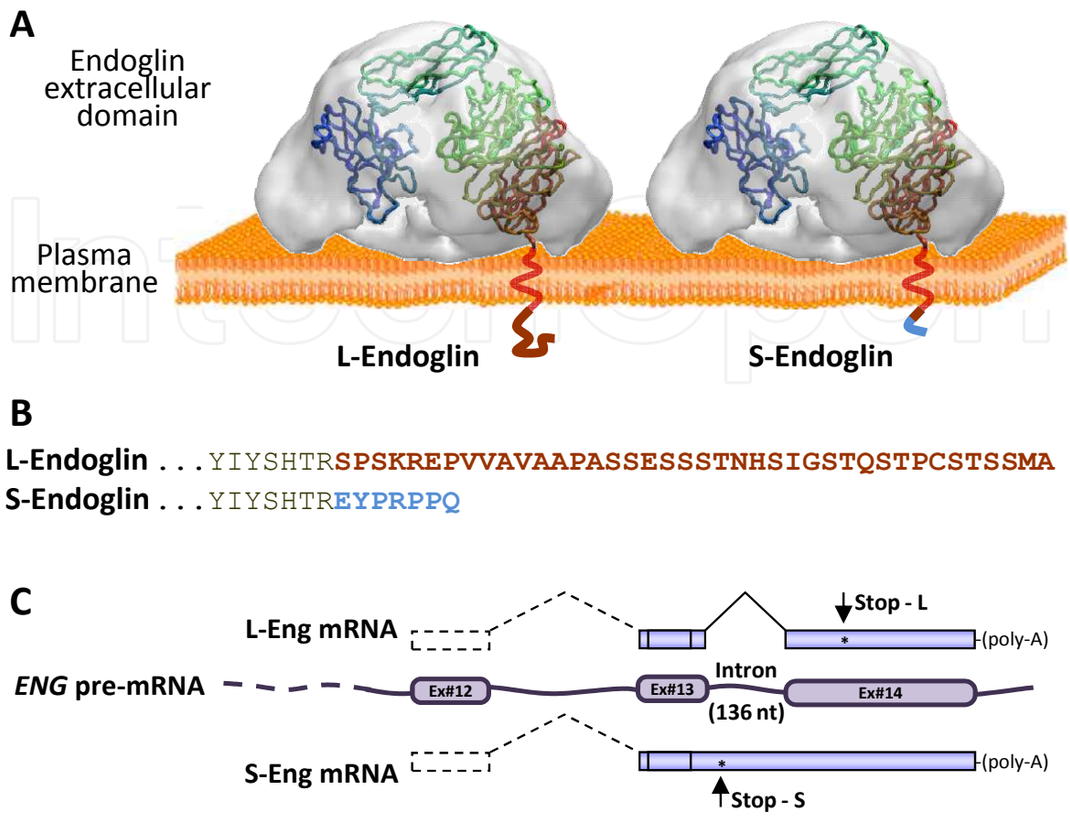


Fig. 1. The two endoglin isoforms. (A) The electron microscopy density map (grey) of the endoglin extracellular region shows the overall structure. The backbone of a theoretical atomic model of the endoglin monomer is fitted inside (adapted from Llorca et al., 2007). This structure is common to both 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).

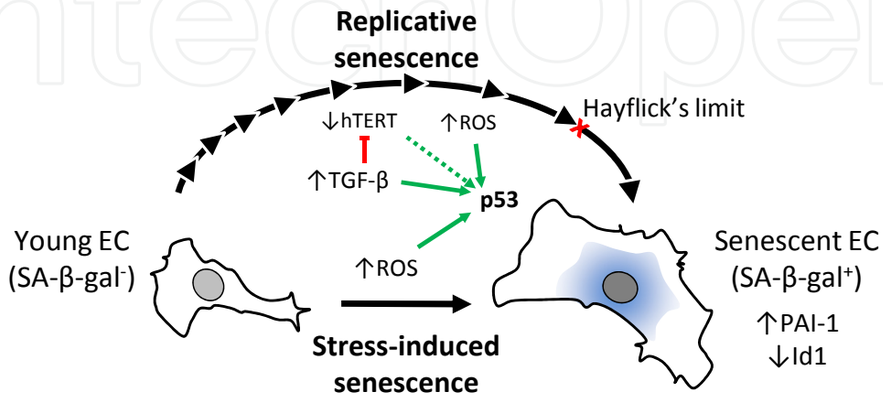


Fig. 2. The endothelial senescence. Endothelial cells extensively cultured *in vitro* enlarge their size and shape, showing a positive blue staining for the SA-β-gal activity. Endothelial senescence is reached by, at least, two different routes, including replicative or oxidative stress-induced. Both 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).

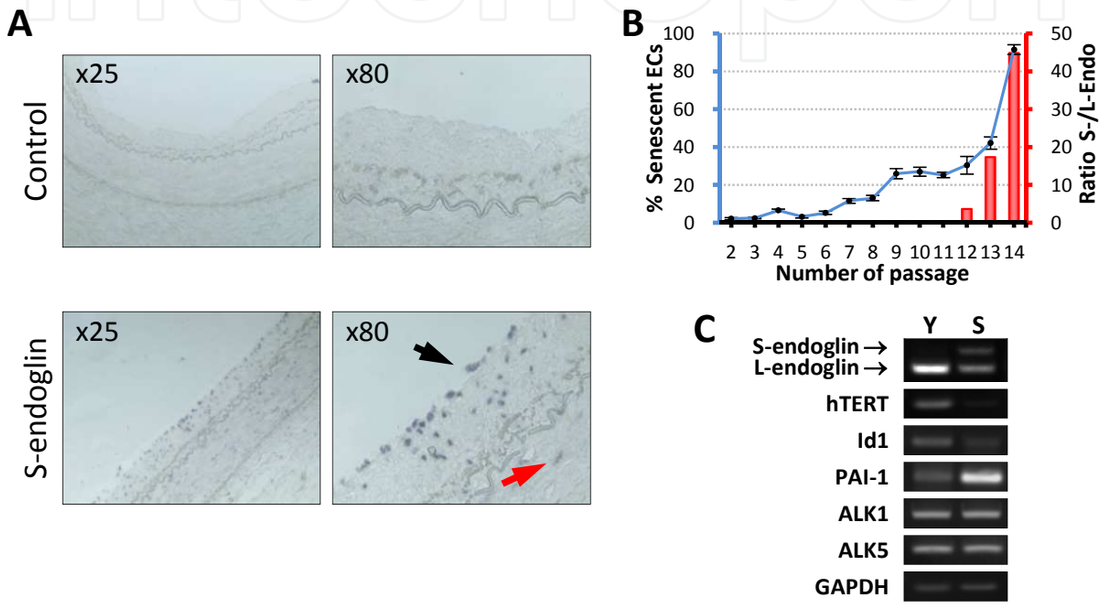


Fig. 3. S-endoglin expression in senescence. (A) The expression of S-endoglin in blood vessels can be revealed by *in situ* hybridization in the endothelium of human coronary artery (black arrow) and in some smooth muscle cells (red arrow). (B) The increase in the percentage of senescent endothelial 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).

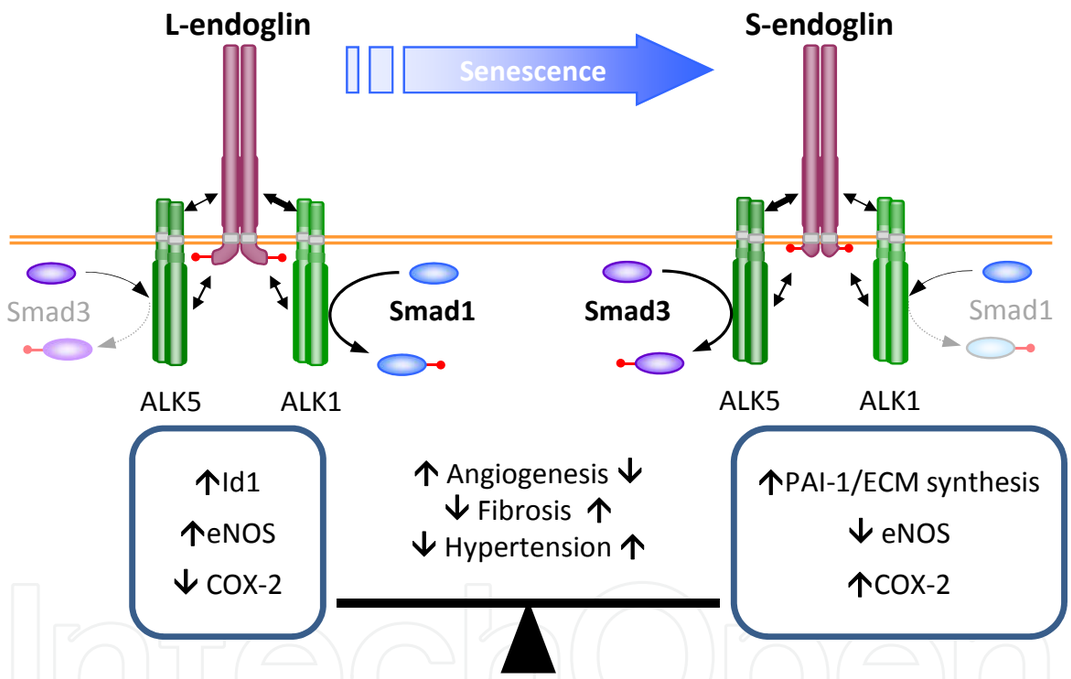


Fig. 4. Functional effects of S-endoglin in endothelial senescence. The S-endoglin up-regulation in aged endothelial cells promotes the ALK5/Smad3 signalling pathway. As a consequent, the vascular physiology is affected decreasing the angiogenesis, increasing the fibrosis and unbalancing the eNOS/COX-2 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).

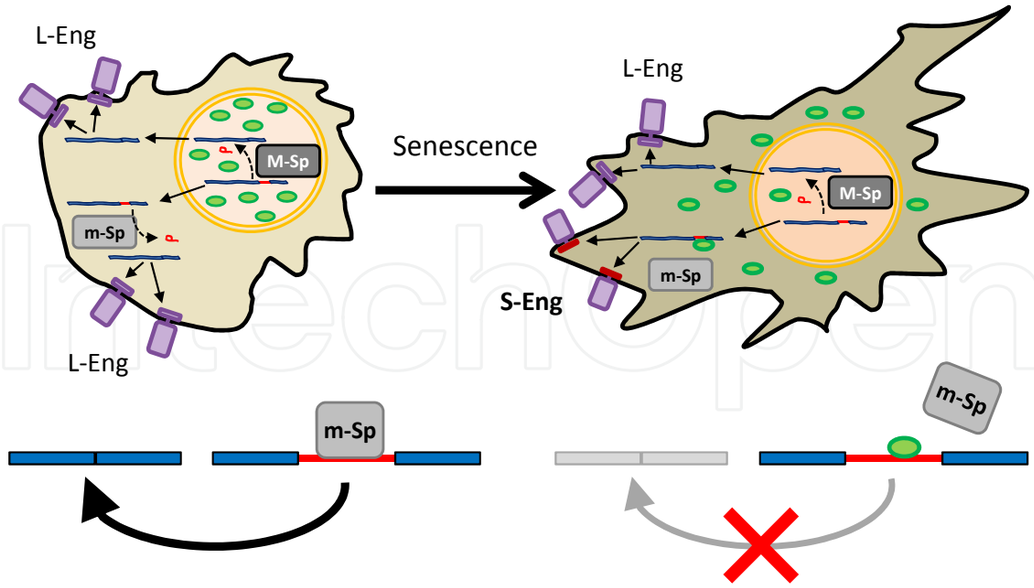


Fig. 5. Regulation of the alternative splicing of endoglin in senescent endothelial cells. In this hypothetical model, the last intron of the *ENG* gene is eliminated in the mature mRNA, so that L-endoglin is the predominantly expressed isoform. In this mRNA processing, both spliceosomes (nuclear M-Sp 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

This work was supported by grants of the Spanish Ministry of Science and Innovation to CB (SAF2010-19222) and *Genoma España* (MEICA). FJB is a post-doctoral researcher of the *Centro de Investigación Biomédica en Red* (CIBER) *de Enfermedades Raras*, ISCIII, Spain. CB is a research professor in the *Centro de Investigaciones Biológicas*, CSIC, Spain.

7. References

Bellon, T.; Corbi, A.; Lastres, P.; Cales, C.; Cebrian, M.; Vera, S.; Cheifetz, S.; Massague, J.; Letarte, M. & Bernabeu, C. (1993). Identification and expression of two forms of the human transforming growth factor-beta-binding protein endoglin with distinct cytoplasmic regions. *European Journal of Immunology*, Vol. 23, No. 9, (September 1993), pp. 2340-2345, ISSN 0014-2980.

Benezra, R.; Davis, R.L.; Lockshon, D.; Turner, D.L. & Weintraub, H. (1990). The protein Id: a negative regulator of helix-loop-helix DNA binding proteins. *Cell*, Vol. 61, No. 1, (April 1990), pp. 49-59, ISSN 0092-8674.

Bernabeu, C.; Lopez-Novoa, J.M. & Quintanilla, M. (2009). The emerging role of TGF-beta superfamily coreceptors in cancer. *Biochimica et Biophysica Acta*, Vol. 1792, No. 10, (July 2009), pp. 954-973, ISSN 0006-3002.

Blanco, F.J. & Bernabeu, C. (2011). Alternative splicing factor or splicing factor-2 plays a key role in intron retention of the endoglin gene during endothelial senescence. *Aging Cell*, Vol. 10, No. 5, (June 2011), pp. 896-907, ISSN 1474-9726.

Blanco, F.J.; Grande, M.T.; Langa, C.; Oujo, B.; Velasco, S.; Rodriguez-Barbero, A.; Perez-Gomez, E.; Quintanilla, M.; Lopez-Novoa, J.M. & Bernabeu, C. (2008). S-endoglin expression is induced in senescent endothelial cells and contributes to vascular pathology. *Circulation Research*, Vol. 103, No. 12, (November 2008), pp. 1383-1392, ISSN 1524-4571.

Blanco, F.J.; Santibanez, J.F.; Guerrero-Esteo, M.; Langa, C.; Vary, C.P. & Bernabeu, C. (2005). Interaction and functional interplay between endoglin and ALK-1, two components

of the endothelial transforming growth factor-beta receptor complex. *Journal of Cellular Physiology*, Vol. 204, No. 2, (February 2005), pp. 574-584, ISSN 0021-9541.

Brandes, R.P.; Fleming, I. & Busse, R. (2005). Endothelial aging. *Cardiovascular Research*, Vol. 66, No. 2, (May 2005), pp. 286-294, ISSN 0008-6363.

Burge, C.; Tuschl, T. & Sharp, P. (1999). Splicing of precursors to mRNAs by the spliceosoomes, In: *The RNA World*. R. Gesteland, T. Cech, and J. Atkins (eds.), pp. 525-560. Cold Spring Harbor Laboratory Press, ISBN 0-87969-589-7, Plainview, NY, United States of America.

Caceres, J.F. & Misteli, T. (2007). Division of labor: minor splicing in the cytoplasm. *Cell*, Vol. 131, No. 4, (November 2007), pp. 645-647, ISSN 0092-8674.

Campisi, J. & d'Adda di Fagagna, F. (2007). Cellular senescence: when bad things happen to good cells. *Nature Reviews Molecular Cell Biology*, Vol. 8, No. 9, (August 2007), pp. 729-740, ISSN 1471-0080.

Carmeliet, P. & Jain, R.K. (2011). Molecular mechanisms and clinical applications of angiogenesis. *Nature*, Vol. 473, No. 7347, (May 2011), pp. 298-307, ISSN 1476-4687.

Cipriano, R.; Kan, C.E.; Graham, J.; Danielpour, D.; Stampfer, M. & Jackson, M.W. (2011). TGF-beta signaling engages an ATM-CHK2-p53-independent RAS-induced senescence and prevents malignant transformation in human mammary epithelial cells. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 108, No. 21, (May 2011), pp. 8668-8673, ISSN 1091-6490.

Collins, C. & Tzima, E. (2011). Hemodynamic forces in endothelial dysfunction and vascular aging. *Experimental Gerontology*, Vol. 46, No. 2-3, (October 2010), pp. 185-188, ISSN 1873-6815.

Comi, P.; Chiaramonte, R. & Maier, J.A. (1995). Senescence-dependent regulation of type 1 plasminogen activator inhibitor in human vascular endothelial cells. *Experimental Cell Research*, Vol. 219, No. 1, (July 1995), pp. 304-308, ISSN 0014-4827.

Conway, E.M. & Carmeliet, P. (2004). The diversity of endothelial cells: a challenge for therapeutic angiogenesis. *Genome Biology*, Vol. 5, No. 2, (January 2004), pp. 207, ISSN 1465-6914.

Chang, M.W.; Grillari, J.; Mayrhofer, C.; Fortschegger, K.; Allmaier, G.; Marzban, G.; Katinger, H. & Voglauer, R. (2005). Comparison of early passage, senescent and hTERT immortalized endothelial cells. *Experimental Cell Research*, Vol. 309, No. 1, (June 2005), pp. 121-136, ISSN 0014-4827.

Chen, J. & Goligorsky, M.S. (2006). Premature senescence of endothelial cells: Methusaleh's dilemma. *American Journal of Physiology. Heart and Circulatory Physiology*, Vol. 290, No. 5, (April 2006), pp. H1729-1739, ISSN 0363-6135.

Chen, Q.M.; Bartholomew, J.C.; Campisi, J.; Acosta, M.; Reagan, J.D. & Ames, B.N. (1998). Molecular analysis of H2O2-induced senescent-like growth arrest in normal human fibroblasts: p53 and Rb control G1 arrest but not cell replication. *Biochemical Journal*, Vol. 332 (Pt 1), (May 1998), pp. 43-50, ISSN 0264-6021.

Chen, S.; Townsend, K.; Goldberg, T.E.; Davies, P. & Conejero-Goldberg, C. (2010). MAPT isoforms: differential transcriptional profiles related to 3R and 4R splice variants. *Journal of Alzheimer's Disease*, Vol. 22, No. 4, (October 2010), pp. 1313-1329, ISSN 1875-8908.

Debacq-Chainiaux, F.; Borlon, C.; Pascal, T.; Royer, V.; Eliaers, F.; Ninane, N.; Carrard, G.; Friguet, B.; de Longueville, F.; Boffe, S.; Remacle, J. & Toussaint, O. (2005).

Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-beta1 signaling pathway. *Journal of Cell Science*, Vol. 118, No. 4, (January 2005), pp. 743-758, ISSN 0021-9533.

Dimri, G.P.; Lee, X.; Basile, G.; Acosta, M.; Scott, G.; Roskelley, C.; Medrano, E.E.; Linskens, M.; Rubelj, I.; Pereira-Smith, O. & et al. (1995). A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 92, No. 20, (September 1995), pp. 9363-9367, ISSN 0027-8424.

Eriksson, M.; Brown, W.T.; Gordon, L.B.; Glynn, M.W.; Singer, J.; Scott, L.; Erdos, M.R.; Robbins, C.M.; Moses, T.Y.; Berglund, P.; Dutra, A.; Pak, E.; Durkin, S.; Csoka, A.B.; Boehnke, M.; Glover, T.W. & Collins, F.S. (2003). Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature*, Vol. 423, No. 6937, (April 2003), pp. 293-298, ISSN 0028-0836.

Erusalimsky, J.D. (2009). Vascular endothelial senescence: from mechanisms to pathophysiology. *Journal of Applied Physiology*, Vol. 106, No. 1, (November 2008), pp. 326-332, ISSN 8750-7587.

Erusalimsky, J.D. & Kurz, D.J. (2005). Cellular senescence in vivo: its relevance in ageing and cardiovascular disease. *Experimental Gerontology*, Vol. 40, No. 8-9, (June 2005), pp. 634-642, ISSN 0531-5565.

Erusalimsky, J.D. & Skene, C. (2009). Mechanisms of endothelial senescence. *Experimental Physiology*, Vol. 94, No. 3, (October 2008), pp. 299-304, ISSN 1469-445X.

Ewald, J.A.; Desotelle, J.A.; Wilding, G. & Jarrard, D.F. (2010). Therapy-induced senescence in cancer. *Journal of the National Cancer Institute*, Vol. 102, No. 20, (September 2010), pp. 1536-1546, ISSN 1460-2105.

Ferrari, A.U.; Radaelli, A. & Centola, M. (2003). Invited review: aging and the cardiovascular system. *Journal of Applied Physiology*, Vol. 95, No. 6, (December 2003), pp. 2591-2597, ISSN 8750-7587.

Folini, M.; Venturini, L.; Cimino-Reale, G. & Zaffaroni, N. (2011). Telomeres as targets for anticancer therapies. *Expert Opinion on Therapeutic Targets*, Vol. 15, No. 5, (February 2011), pp. 579-593, ISSN 1744-7631.

Foreman, K.E. & Tang, J. (2003). Molecular mechanisms of replicative senescence in endothelial cells. *Experimental Gerontology*, Vol. 38, No. 11-12, (November 2003), pp. 1251-1257, ISSN 0531-5565.

Foteinos, G.; Hu, Y.; Xiao, Q.; Metzler, B. & Xu, Q. (2008). Rapid endothelial turnover in atherosclerosis-prone areas coincides with stem cell repair in apolipoprotein E-deficient mice. *Circulation*, Vol. 117, No. 14, (April 2008), pp. 1856-1863, ISSN 1524-4539.

Fraisl, P.; Mazzone, M.; Schmidt, T. & Carmeliet, P. (2009). Regulation of angiogenesis by oxygen and metabolism. *Developmental Cell*, Vol. 16, No. 2, (February 2009), pp. 167-179, ISSN 1878-1551.

Fujiki, T.; Miura, T.; Maura, M.; Shiraishi, H.; Nishimura, S.; Imada, Y.; Uehara, N.; Tashiro, K.; Shirahata, S. & Katakura, Y. (2007). TAK1 represses transcription of the human telomerase reverse transcriptase gene. *Oncogene*, Vol. 26, No. 36, (February 2007), pp. 5258-5266, ISSN 0950-9232.

Furukawa, A.; Tada-Oikawa, S.; Kawanishi, S. & Oikawa, S. (2007). H2O2 accelerates cellular senescence by accumulation of acetylated p53 via decrease in the function

of SIRT1 by NAD+ depletion. *Cellular Physiology and Biochemistry*, Vol. 20, No. 1-4, (June 2007), pp. 45-54, ISSN 1015-8987.

Ghosh, A.K. & Vaughan, D.E. (2011). PAI-1 in Tissue Fibrosis. *Journal of Cellular Physiology*, (April 2011), DOI: 10.1002/jcp.22783, ISSN 1097-4652.

Graveley, B.R. (2000). Sorting out the complexity of SR protein functions. *RNA*, Vol. 6, No. 9, (September 2000), pp. 1197-1211, ISSN 1355-8382.

Graveley, B.R. (2001). Alternative splicing: increasing diversity in the proteomic world. *Trends in Genetics*, Vol. 17, No. 2, (February 2001), pp. 100-107, ISSN 0168-9525.

Grisham, M.B.; Granger, D.N. & Lefer, D.J. (1998). Modulation of leukocyte-endothelial interactions by reactive metabolites of oxygen and nitrogen: relevance to ischemic heart disease. *Free Radical Biology & Medicine*, Vol. 25, No. 4-5, (September 1998), pp. 404-433, ISSN 0891-5849.

Hall, S.L. & Padgett, R.A. (1996). Requirement of U12 snRNA for in vivo splicing of a minor class of eukaryotic nuclear pre-mRNA introns. *Science*, Vol. 271, No. 5256, (March 1996), pp. 1716-1718, ISSN 0036-8075.

Harries, L.W.; Hernandez, D.; Henley, W.; Wood, A.R.; Holly, A.C.; Bradley-Smith, R.M.; Yaghootkar, H.; Dutta, A.; Murray, A.; Frayling, T.M.; Guralnik, J.M.; Bandinelli, S.; Singleton, A.; Ferrucci, L. & Melzer, D. (2011). Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing. *Aging Cell*, Vol. 10, No. 5, (June 2011), pp. 868-878, ISSN 1474-9726.

Hayashi, T.; Matsui-Hirai, H.; Miyazaki-Akita, A.; Fukatsu, A.; Funami, J.; Ding, Q.F.; Kamalanathan, S.; Hattori, Y.; Ignarro, L.J. & Iguchi, A. (2006). Endothelial cellular senescence is inhibited by nitric oxide: implications in atherosclerosis associated with menopause and diabetes. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 103, No. 45, (November 2006), pp. 17018-17023, ISSN 0027-8424.

Hayflick, L. (2003). Living forever and dying in the attempt. *Experimental Gerontology*, Vol. 38, No. 11-12, (December 2003), pp. 1231-1241, ISSN 0531-5565.

Imaizumi, N.; Monnier, Y.; Hegi, M.; Mirimanoff, R.O. & Ruegg, C. (2010). Radiotherapy suppresses angiogenesis in mice through TGF-betaRI/ALK5-dependent inhibition of endothelial cell sprouting. *PLoS One*, Vol. 5, No. 6, (June 2010), pp. e11084, ISSN 1932-6203.

Jerkic, M.; Rivas-Elena, J.V.; Santibanez, J.F.; Prieto, M.; Rodriguez-Barbero, A.; Perez-Barriocanal, F.; Pericacho, M.; Arevalo, M.; Vary, C.P.; Letarte, M.; Bernabeu, C. & Lopez-Novoa, J.M. (2006). Endoglin regulates cyclooxygenase-2 expression and activity. *Circulation Research*, Vol. 99, No. 3, (July 2006), pp. 248-256, ISSN 1524-4571.

Jurica, M.S. & Moore, M.J. (2003). Pre-mRNA splicing: awash in a sea of proteins. *Molecular Cell*, Vol. 12, No. 1, (July 2003), pp. 5-14, ISSN 1097-2765.

Kang, J.S.; Liu, C. & Derynck, R. (2009). New regulatory mechanisms of TGF-beta receptor function. *Trends in Cell Biology*, Vol. 19, No. 8, (August 2009), pp. 385-394, ISSN 1879-3088.

Kang, Y.; Chen, C.R. & Massague, J. (2003). A self-enabling TGFbeta response coupled to stress signaling: Smad engages stress response factor ATF3 for Id1 repression in epithelial cells. *Molecular Cell*, Vol. 11, No. 4, (April 2003), pp. 915-926, ISSN 1097-2765.

Kao, C.L.; Chen, L.K.; Chang, Y.L.; Yung, M.C.; Hsu, C.C.; Chen, Y.C.; Lo, W.L.; Chen, S.J.; Ku, H.H. & Hwang, S.J. (2010). Resveratrol protects human endothelium from H(2)O(2)-induced oxidative stress and senescence via SirT1 activation. *Journal of Atherosclerosis and Thrombosis*, Vol. 17, No. 9, (July 2010), pp. 970-979, ISSN 1880-3873.

Kim, K.S.; Kang, K.W.; Seu, Y.B.; Baek, S.H. & Kim, J.R. (2009). Interferon-gamma induces cellular senescence through p53-dependent DNA damage signaling in human endothelial cells. *Mechanisms of Ageing and Development*, Vol. 130, No. 3, (December 2008), pp. 179-188, ISSN 0047-6374.

Koleva, R.I.; Conley, B.A.; Romero, D.; Riley, K.S.; Marto, J.A.; Lux, A. & Vary, C.P. (2006). Endoglin structure and function: Determinants of endoglin phosphorylation by transforming growth factor-beta receptors. *Journal of Biological Chemistry*, Vol. 281, No. 35, (June 2006), pp. 25110-25123, ISSN 0021-9258.

Konig, H.; Matter, N.; Bader, R.; Thiele, W. & Muller, F. (2007). Splicing segregation: the minor spliceosome acts outside the nucleus and controls cell proliferation. *Cell*, Vol. 131, No. 4, (November 2007), pp. 718-729, ISSN 0092-8674.

Kordon, E.C.; McKnight, R.A.; Jhappan, C.; Hennighausen, L.; Merlino, G. & Smith, G.H. (1995). Ectopic TGF beta 1 expression in the secretory mammary epithelium induces early senescence of the epithelial stem cell population. *Developmental Biology*, Vol. 168, No. 1, (March 1995), pp. 47-61, ISSN 0012-1606.

Kortlever, R.M.; Nijwening, J.H. & Bernards, R. (2008). Transforming growth factor-beta requires its target plasminogen activator inhibitor-1 for cytostatic activity. *Journal of Biological Chemistry*, Vol. 283, No. 36, (July 2008), pp. 24308-24313, ISSN 0021-9258.

Kwan, T.; Benovoy, D.; Dias, C.; Gurd, S.; Serre, D.; Zuzan, H.; Clark, T.A.; Schweitzer, A.; Staples, M.K.; Wang, H.; Blume, J.E.; Hudson, T.J.; Sladek, R. & Majewski, J. (2007). Heritability of alternative splicing in the human genome. *Genome Research*, Vol. 17, No. 8, (August 2007), pp. 1210-1218, ISSN 1088-9051.

Lareau, L.F.; Green, R.E.; Bhatnagar, R.S. & Brenner, S.E. (2004). The evolving roles of alternative splicing. *Current Opinion in Structural Biology*, Vol. 14, No. 3, (June 2004), pp. 273-282, ISSN 0959-440X.

Lebrin, F.; Goumans, M.J.; Jonker, L.; Carvalho, R.L.; Valdimarsdottir, G.; Thorikay, M.; Mummery, C.; Arthur, H.M. & ten Dijke, P. (2004). Endoglin promotes endothelial cell proliferation and TGF-beta/ALK1 signal transduction. *The EMBO Journal*, Vol. 23, No. 20, (September 2004), pp. 4018-4028, ISSN 0261-4189.

Li, H. & Liu, J.P. (2007). Mechanisms of action of TGF-beta in cancer: evidence for Smad3 as a repressor of the hTERT gene. *Annals of the New York Academy of Sciences*, Vol. 1114, (October 2007), pp. 56-68, ISSN 0077-8923.

Li, H.; Xu, D.; Li, J.; Berndt, M.C. & Liu, J.P. (2006). Transforming growth factor beta suppresses human telomerase reverse transcriptase (hTERT) by Smad3 interactions with c-Myc and the hTERT gene. *Journal of Biological Chemistry*, Vol. 281, No. 35, (June 2006), pp. 25588-25600, ISSN 0021-9258.

Lopez-Novoa, J.M. & Bernabeu, C. (2010). The physiological role of endoglin in the cardiovascular system. *American Journal of Physiology. Heart and Circulatory Physiology*, Vol. 299, No. 4, (July 2010), pp. H959-974, ISSN 1522-1539.

Llorca, O.; Trujillo, A.; Blanco, F.J. & Bernabeu, C. (2007). Structural model of human endoglin, a transmembrane receptor responsible for hereditary hemorrhagic

telangiectasia. *Journal of Molecular Biology*, Vol. 365, No. 3, (November 2006), pp. 694-705, ISSN 0022-2836.

Mahmoud, M.; Allinson, K.R.; Zhai, Z.; Oakenfull, R.; Ghandi, P.; Adams, R.H.; Fruttiger, M. & Arthur, H.M. (2010). Pathogenesis of arteriovenous malformations in the absence of endoglin. *Circulation Research*, Vol. 106, No. 8, (March 2010), pp. 1425-1433, ISSN 1524-4571.

Massague, J.; Seoane, J. & Wotton, D. (2005). Smad transcription factors. *Genes & Development*, Vol. 19, No. 23, (December 2005), pp. 2783-2810, ISSN 0890-9369.

Matlin, A.J.; Clark, F. & Smith, C.W. (2005). Understanding alternative splicing: towards a cellular code. *Nature Reviews Molecular Cell Biology*, Vol. 6, No. 5, (June 2005), pp. 386-398, ISSN 1471-0072.

McAllister, K.A.; Grogg, K.M.; Johnson, D.W.; Gallione, C.J.; Baldwin, M.A.; Jackson, C.E.; Helmbold, E.A.; Markel, D.S.; McKinnon, W.C.; Murrell, J. & et al. (1994). Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. *Nature Genetics*, Vol. 8, No. 4, (December 1994), pp. 345-351, ISSN 1061-4036.

Meshorer, E. & Soreq, H. (2002). Pre-mRNA splicing modulations in senescence. *Aging Cell*, Vol. 1, No. 1, (October 2002), pp. 10-16, ISSN 1474-9718.

Meurer, S.K.; Tihaa, L.; Borkham-Kamphorst, E. & Weiskirchen, R. (2011). Expression and functional analysis of endoglin in isolated liver cells and its involvement in fibrogenic Smad signalling. *Cellular Signalling*, Vol. 23, No. 4, (December 2010), pp. 683-699, ISSN 1873-3913.

Minamino, T. & Komuro, I. (2007). Vascular cell senescence: contribution to atherosclerosis. *Circulation Research*, Vol. 100, No. 1, (January 2007), pp. 15-26, ISSN 1524-4571.

Minamino, T. & Komuro, I. (2008). Role of telomeres in vascular senescence. *Frontiers in Bioscience*, Vol. 13, (January 2008), pp. 2971-2979, ISSN 1093-4715.

Minamino, T.; Miyauchi, H.; Yoshida, T.; Tateno, K.; Kunieda, T. & Komuro, I. (2004). Vascular cell senescence and vascular aging. *Journal of Molecular and Cellular Cardiology*, Vol. 36, No. 2, (February 2004), pp. 175-183, ISSN 0022-2828.

Moore, M.J. & Silver, P.A. (2008). Global analysis of mRNA splicing. *RNA*, Vol. 14, No. 2, (December 2007), pp. 197-203, ISSN 1469-9001.

Nott, A.; Meislin, S.H. & Moore, M.J. (2003). A quantitative analysis of intron effects on mammalian gene expression. *RNA*, Vol. 9, No. 5, (April 2003), pp. 607-617, ISSN 1355-8382.

Nowak, D.G.; Amin, E.M.; Rennel, E.S.; Hoareau-Aveilla, C.; Gammons, M.; Damodoran, G.; Hagiwara, M.; Harper, S.J.; Woolard, J.; Ladomery, M.R. & Bates, D.O. (2010). Regulation of vascular endothelial growth factor (VEGF) splicing from pro-angiogenic to anti-angiogenic isoforms: a novel therapeutic strategy for angiogenesis. *Journal of Biological Chemistry*, Vol. 285, No. 8, (November 2009), pp. 5532-5540, ISSN 1083-351X.

Nowak, D.G.; Woolard, J.; Amin, E.M.; Konopatskaya, O.; Saleem, M.A.; Churchill, A.J.; Ladomery, M.R.; Harper, S.J. & Bates, D.O. (2008). Expression of pro- and anti-angiogenic isoforms of VEGF is differentially regulated by splicing and growth factors. *Journal of Cell Science*, Vol. 121, No. Pt 20, (October 2008), pp. 3487-3495, ISSN 0021-9533.

Pardali, E.; Goumans, M.J. & ten Dijke, P. (2010). Signaling by members of the TGF-beta family in vascular morphogenesis and disease. *Trends in Cell Biology*, Vol. 20, No. 9, (September 2010), pp. 556-567, ISSN 1879-3088.

Paris, F.; Fuks, Z.; Kang, A.; Capodieci, P.; Juan, G.; Ehleiter, D.; Haimovitz-Friedman, A.; Cordon-Cardo, C. & Kolesnick, R. (2001). Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. *Science*, Vol. 293, No. 5528, (July 2001), pp. 293-297, ISSN 0036-8075.

Pascual, G.; Mendieta, C.; Garcia-Honduvilla, N.; Corrales, C.; Bellon, J.M. & Bujan, J. (2007). TGF-beta1 upregulation in the aging varicose vein. *Journal of Vascular Research*, Vol. 44, No. 3, (March 2007), pp. 192-201, ISSN 1018-1172.

Perez-Gomez, E.; Del Castillo, G.; Santibanez, J.F.; Lopez-Novoa, J.M.; Bernabeu, C. & Quintanilla, M. (2010). The role of the TGF-beta coreceptor endoglin in cancer. *The Scientific World Journal*, Vol. 10, (December 2010), pp. 2367-2384, ISSN 1537-744X.

Perez-Gomez, E.; Eleno, N.; Lopez-Novoa, J.M.; Ramirez, J.R.; Velasco, B.; Letarte, M.; Bernabeu, C. & Quintanilla, M. (2005). Characterization of murine S-endoglin isoform and its effects on tumor development. *Oncogene*, Vol. 24, No. 27, (April 2005), pp. 4450-4461, ISSN 0950-9232.

Rivard, A.; Fabre, J.E.; Silver, M.; Chen, D.; Murohara, T.; Kearney, M.; Magner, M.; Asahara, T. & Isner, J.M. (1999). Age-dependent impairment of angiogenesis. *Circulation*, Vol. 99, No. 1, (January 1999), pp. 111-120, ISSN 0009-7322.

Rodríguez-Mañas, L.; El-Assar, M.; Vallejo, S.; López-Dóriga, P.; Solís, J.; Petidier, R.; Montes, M.; Nevado, J.; Castro, M.; Gómez-Guerrero, C.; Peiró, C. & Sánchez-Ferrer, C.F. (2009). Endothelial dysfunction in aged humans is related with oxidative stress and vascular inflammation. *Aging Cell*, Vol. 8, No. 3, (June 2009), pp. 226-238, ISSN 1474-9726.

Rosso, A.; Balsamo, A.; Gambino, R.; Dentelli, P.; Falcioni, R.; Cassader, M.; Pegoraro, L.; Pagano, G. & Brizzi, M.F. (2006). p53 Mediates the accelerated onset of senescence of endothelial progenitor cells in diabetes. *Journal of Biological Chemistry*, Vol. 281, No. 7, (December 2005), pp. 4339-4347, ISSN 0021-9258.

Sakabe, N.J. & de Souza, S.J. (2007). Sequence features responsible for intron retention in human. *BMC Genomics*, Vol. 8, (February 2007), pp. 59, ISSN 1471-2164.

Sanford, J.R.; Coutinho, P.; Hackett, J.A.; Wang, X.; Ranahan, W. & Caceres, J.F. (2008). Identification of nuclear and cytoplasmic mRNA targets for the shuttling protein SF2/ASF. *PLoS One*, Vol. 3, No. 10, (October 2008), pp. e3369, ISSN 1932-6203.

Sanford, J.R.; Ellis, J.D.; Cazalla, D. & Caceres, J.F. (2005). Reversible phosphorylation differentially affects nuclear and cytoplasmic functions of splicing factor 2/alternative splicing factor. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 102, No. 42, (October 2005), pp. 15042-15047, ISSN 0027-8424.

Santibanez, J.F.; Letamendia, A.; Perez-Barriocanal, F.; Silvestri, C.; Saura, M.; Vary, C.P.; Lopez-Novoa, J.M.; Attisano, L. & Bernabeu, C. (2007). Endoglin increases eNOS expression by modulating Smad2 protein levels and Smad2-dependent TGF-beta signaling. *Journal of Cellular Physiology*, Vol. 210, No. 2, (October 2006), pp. 456-468, ISSN 0021-9541.

Saura, M.; Zaragoza, C.; Herranz, B.; Griera, M.; Diez-Marques, L.; Rodriguez-Puyol, D. & Rodriguez-Puyol, M. (2005). Nitric oxide regulates transforming growth factor-beta

signaling in endothelial cells. *Circulation Research*, Vol. 97, No. 11, (October 2005), pp. 1115-1123, ISSN 1524-4571.

Schneiderman, J.; Sawdey, M.S.; Keeton, M.R.; Bordin, G.M.; Bernstein, E.F.; Dilley, R.B. & Loskutoff, D.J. (1992). Increased type 1 plasminogen activator inhibitor gene expression in atherosclerotic human arteries. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 89, No. 15, (August 1992), pp. 6998-7002, ISSN 0027-8424.

Shay, J.W. & Wright, W.E. (2007). Hallmarks of telomeres in ageing research. *Journal of Pathology*, Vol. 211, No. 2, (January 2007), pp. 114-123, ISSN 0022-3417.

Sheth, N.; Roca, X.; Hastings, M.L.; Roeder, T.; Krainer, A.R. & Sachidanandam, R. (2006). Comprehensive splice-site analysis using comparative genomics. *Nucleic Acids Research*, Vol. 34, No. 14, (August 2006), pp. 3955-3967, ISSN 1362-4962.

Shovlin, C.L. (2010). Hereditary haemorrhagic telangiectasia: pathophysiology, diagnosis and treatment. *Blood Reviews*, Vol. 24, No. 6, (September 2010), pp. 203-219, ISSN 1532-1681.

Singh, R. & Valcarcel, J. (2005). Building specificity with nonspecific RNA-binding proteins. *Nature Structure Molecular Biology*, Vol. 12, No. 8, (August 2005), pp. 645-653, ISSN 1545-9993.

Sinha, R.; Allemand, E.; Zhang, Z.; Karni, R.; Myers, M.P. & Krainer, A.R. (2010). Arginine methylation controls the subcellular localization and functions of the oncoprotein splicing factor SF2/ASF. *Molecular Cell Biology*, Vol. 30, No. 11, (March 2010), pp. 2762-2774, ISSN 1098-5549.

Sperling, J.; Azubel, M. & Sperling, R. (2008). Structure and function of the Pre-mRNA splicing machine. *Structure*, Vol. 16, No. 11, (November 2008), pp. 1605-1615, ISSN 0969-2126.

Steiner, D.R.; Gonzalez, N.C. & Wood, J.G. (2002). Interaction between reactive oxygen species and nitric oxide in the microvascular response to systemic hypoxia. *Journal of Applied Physiology*, Vol. 93, No. 4, (September 2002), pp. 1411-1418, ISSN 8750-7587.

Sugrue, M.M.; Shin, D.Y.; Lee, S.W. & Aaronson, S.A. (1997). Wild-type p53 triggers a rapid senescence program in human tumor cells lacking functional p53. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 94, No. 18, (September 1997), pp. 9648-9653, ISSN 0027-8424.

Sun, D.; Huang, A.; Yan, E.H.; Wu, Z.; Yan, C.; Kaminski, P.M.; Oury, T.D.; Wolin, M.S. & Kaley, G. (2004). Reduced release of nitric oxide to shear stress in mesenteric arteries of aged rats. *American Journal of Physiology. Heart and Circulatory Physiology*, Vol. 286, No. 6, (January 2004), pp. H2249-2256, ISSN 0363-6135.

Taddei, S.; Virdis, A.; Ghiadoni, L.; Salvetti, G.; Bernini, G.; Magagna, A. & Salvetti, A. (2001). Age-related reduction of NO availability and oxidative stress in humans. *Hypertension*, Vol. 38, No. 2, (August 2001), pp. 274-279, ISSN 1524-4563.

Tang, J.; Gordon, G.M.; Nickoloff, B.J. & Foreman, K.E. (2002). The helix-loop-helix protein id-1 delays onset of replicative senescence in human endothelial cells. *Laboratory Investigation*, Vol. 82, No. 8, (August 2002), pp. 1073-1079, ISSN 0023-6837.

Tang, Y.; Yang, X.; Friesel, R.E.; Vary, C.P. & Liaw, L. (2011). Mechanisms of TGF-beta-Induced Differentiation in Human Vascular Smooth Muscle Cells. *Journal of Vascular Research*, Vol. 48, No. 6, (August 2011), pp. 485-494, ISSN 1423-0135.

Tarn, W.Y. & Steitz, J.A. (1996). Highly diverged U4 and U6 small nuclear RNAs required for splicing rare AT-AC introns. *Science*, Vol. 273, No. 5283, (September 1996), pp. 1824-1832, ISSN 0036-8075.

ten Dijke, P.; Goumans, M.J. & Pardali, E. (2008). Endoglin in angiogenesis and vascular diseases. *Angiogenesis*, Vol. 11, No. 1, (February 2008), pp. 79-89, ISSN 0969-6970.

Toussaint, O.; Medrano, E.E. & von Zglinicki, T. (2000). Cellular and molecular mechanisms of stress-induced premature senescence (SIPS) of human diploid fibroblasts and melanocytes. *Experimental Gerontology*, Vol. 35, No. 8, (December 2000), pp. 927-945, ISSN 0531-5565.

Tremain, R.; Marko, M.; Kinnimulki, V.; Ueno, H.; Bottinger, E. & Glick, A. (2000). Defects in TGF-beta signaling overcome senescence of mouse keratinocytes expressing v-Ha-ras. *Oncogene*, Vol. 19, No. 13, (April 2000), pp. 1698-1709, ISSN 0950-9232.

van der Kraan, P.M.; Goumans, M.J.; Blaney Davidson, E. & Ten Dijke, P. (2011). Age-dependent alteration of TGF-beta signalling in osteoarthritis. *Cell and Tissue Research*, DOI: 10.1007/s00441-011-1194-6, (June 2011), ISSN 1432-0878.

Velasco, S.; Alvarez-Munoz, P.; Pericacho, M.; Dijke, P.T.; Bernabeu, C.; Lopez-Novoa, J.M. & Rodriguez-Barbero, A. (2008). L- and S-endoglin differentially modulate TGFbeta1 signaling mediated by ALK1 and ALK5 in L6E9 myoblasts. *Journal of Cell Science*, Vol. 121, No. Pt 6, (February 2008), pp. 913-919, ISSN 0021-9533.

Venkatesha, S.; Toporsian, M.; Lam, C.; Hanai, J.; Mammoto, T.; Kim, Y.M.; Bdolah, Y.; Lim, K.H.; Yuan, H.T.; Libermann, T.A.; Stillman, I.E.; Roberts, D.; D'Amore, P.A.; Epstein, F.H.; Sellke, F.W.; Romero, R.; Sukhatme, V.P.; Letarte, M. & Karumanchi, S.A. (2006). Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nature Medicine*, Vol. 12, No. 6, (June 2006), pp. 642-649, ISSN 1078-8956.

Voglauer, R.; Chang, M.W.; Dampier, B.; Wieser, M.; Baumann, K.; Sterovsky, T.; Schreiber, M.; Katinger, H. & Grillari, J. (2006). SNEV overexpression extends the life span of human endothelial cells. *Experimental Cell Research*, Vol. 312, No. 6, (January 2006), pp. 746-759, ISSN 0014-4827.

Wang, Z.; Xiao, X.; Van Nostrand, E. & Burge, C.B. (2006). General and specific functions of exonic splicing silencers in splicing control. *Molecular Cell*, Vol. 23, No. 1, (June 2006), pp. 61-70, ISSN 1097-2765.

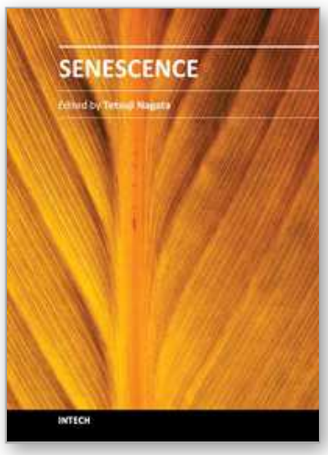
Wesierska-Gadek, J.; Wojciechowski, J.; Ranftler, C. & Schmid, G. (2005). Role of p53 tumor suppressor in ageing: regulation of transient cell cycle arrest and terminal senescence. *Journal of Physiology and Pharmacology*, Vol. 56, No. 1, (March 2005), pp. 15-28, ISSN 0867-5910.

Will, C.L. & Luhrmann, R. (2005). Splicing of a rare class of introns by the U12-dependent spliceosome. *Biological Chemistry*, Vol. 386, No. 8, (October 2005), pp. 713-724, ISSN 1431-6730.

Wu, S.; Hultquist, A.; Hydbring, P.; Cetinkaya, C.; Oberg, F. & Larsson, L.G. (2009). TGF-beta enforces senescence in Myc-transformed hematopoietic tumor cells through induction of Mad1 and repression of Myc activity. *Experimental Cell Research*, Vol. 315, No. 18, (September 2009), pp. 3099-3111, ISSN 1090-2422.

Young, A.R. & Narita, M. (2009). SASP reflects senescence. *EMBO Reports*, Vol. 10, No. 3, (February 2009), pp. 228-230, ISSN 1469-3178.

Zhou, Z.; Licklider, L.J.; Gygi, S.P. & Reed, R. (2002). Comprehensive proteomic analysis of the human spliceosome. *Nature*, Vol. 419, No. 6903, (September 2002), pp. 182-185, ISSN 0028-0836.



Senescence
Edited by Dr. Tetsuji Nagata

ISBN 978-953-51-0144-4
Hard cover, 850 pages
Publisher InTech
Published online 29, February, 2012
Published in print edition February, 2012

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.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Francisco J. Blanco and Carmelo Bernabéu (2012). Alternative Splicing in Endothelial Senescence: Role of the TGF-β Co-Receptor Endoglin, Senescence, Dr. Tetsuji Nagata (Ed.), ISBN: 978-953-51-0144-4, InTech, Available from: http://www.intechopen.com/books/senescence/alternative-splicing-in-endothelial-senescence-role-of-the-tgf-beta-co-receptor-endoglin

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