Title: Is there a Role for Placental Senescence in the Genesis of Obstetrical Complications and Fetal Growth Restriction?

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Glossary of terms:

Telomeres – highly conserved repetitive DNA regions, consist of tandem arrays of the hexanucleotide sequence 'TTAGGG' in the human. Telomeres protect DNA ends from breaks, end-to-end fusion and degradation by forming a protective cap with a guanine-rich single-stranded telomere overhang.

Telomerase – a reverse transcriptase enzyme, which regulates telomere length by adding telomeric repeats to the ends of chromosomes.

Replicative senescence – dependent on telomere length, occurs as a result of progressive telomere shortening during mitotic cell division. DNA polymerases are unable to replicate DNA at the ends of chromosomes (known as the 'end-replication problem' of eukaryote DNA) leaving ~50-200 bp of unreplicated telomeric DNA in each round of DNA replication. When telomeres reach a critical minimum length, their protective structure is distorted (leads to dysfunctional telomeres), resulting in the exposure of DNA ends and a DNA damage response, which leads to the activation of the cellular senescence pathway.

Premature senescence – is independent of telomere length and occurs as a consequence of DNA damage and the DNA damage response caused by stress such as elevated reactive oxygen species, activation of oncogenes, telomere dysfunction and cell-cell fusion.

RAS – a mutant RAS-p21 protein, RAS involves transmitting signals and activating signaling cascades, including mitogen-activated protein kinase (MAPK) and PI3K/mTOR pathways

Chromatin – Chromatin is a mass of genetic material composed of DNA and proteins, primarily histones, which condenses to form chromosomes during eukaryotic cell division. Chromatin compresses the DNA into a compact unit that will be less voluminous and can fit within the nucleus. Histones help to organize DNA into structures called nucleosomes by providing a base on which the DNA can be wrapped around. Post-translational modification to histone proteins which includes methylation, phosphorylation, and acetylation, can cause disruption in chromatin structure.

Heterochromatin – is a chromatin variety in which DNA, which codes inactive genes ("turned off"), is more condensed and associated with structural proteins. Heterochromatin protects chromosome integrity and gene regulation. While DNA, which codes genes that are actively

transcribed ("turned on"), is more loosely packaged and associated with RNA polymerases, referred to as euchromatin.

Cyclin-dependent kinases (CDKs) – a family of multifunctional enzymes that can phosphorylate various protein substrates involved in cell cycle progression.

Cyclin-dependent kinase inhibitors, p16 and p21 – proteins which inhibit cyclin-dependent kinase and are involved in cell cycle arrest at the G1 phase.

p53 – a tumor suppressor gene.

pRB – retinoblastoma protein (pRB) is a tumor suppressor, which plays a pivotal role in the negative control of the cell cycle and in tumor progression. The pRb protein represses gene transcription, by directly binding to the transactivation domain of E2F genes and by binding to the promoter of these genes as a complex with E2F.

E2F – is a group of genes that code transcription factors, such as E2F1 and E2F2, in higher eukaryotes. The E2F family plays a crucial role in the control of cell cycle and action of tumor suppressor proteins. E2F proteins can mediate both cell proliferation and p53-dependent/independent apoptosis. The pRb binds to the E2F1 transcription factor that preventing it from interacting with the cell's transcription machinery.

Anti-apoptotic Bcl-2 – is regulator proteins that regulate cell death via apoptosis, by inhibiting apoptosis (anti-apoptotic).

ERVWE1 – ERVW-1 gene (endogenous retrovirus group W envelope member 1) is an human defective retroviral fusogen found in humans and other primates that encodes the protein syncytin-1. Syncytin-1 is a cell-cell fusion protein, highly expressed in normal placental tissue whose function is most well characterized in placental development.

p38 MAPK – is a member of mitogen-activated protein kinase (MAPK), which mediates a wide variety of cellular behaviours in response to extracellular stimuli.

mTORC1 – a conserved serine/threonine kinase that induces anabolism by regulating protein translation, nucleotide, and lipid biogenesis and inhibits the catabolic process by blocking autophagy.

AMPK - AMP-activated protein kinase (AMPK) plays a key role as a master regulator of cellular energy homeostasis. The kinase is activated in response to stresses that deplete cellular ATP supplies such as low glucose, hypoxia. Cellular stresses that inhibit ATP production or

increase its consumption change the AMP:ATP ratio and activate the pathway. AMPK activation positively regulates signaling pathways that replenish cellular ATP supplies, including fatty acid oxidation and autophagy.

Reactive oxygen species (ROS) – oxygen free radicals that contain one or more unpaired electrons, produced as by-products of mitochondrial respiration and metabolism, and are capable of activating and modulating various signalling pathways, including those involved in cell growth, differentiation, and metabolism. Examples include peroxides, superoxide, hydroxyl radical, and singlet oxygen.

SA-β-gal – senescence-associated beta-galactosidase (SA-β-gal) is a hydrolase enzyme that catalyzes the hydrolysis of β-galactosides into monosaccharides only in senescent cells. Therefore expression of SA-β-gal is considered to be a biomarker of cellular senescence.

8-OHdG – 8-hydroxy-2' -deoxyguanosine (8-OHdG) is an oxidized derivative of deoxyguanosine. In genomic and mitochondrial DNA, 8-OHdG is one of the major product of free radical-induced oxidative lesions, and has therefore been widely used as a biomarker for DNA damage and oxidative stress.

Mitochondrial fusion and fision – Mitochondria are dynamic organelles that constantly fuse (fusion) and divide (fission) and are termed mitochondrial dynamics. Mitochondria fusion and fission are important for mitochondrial inheritance and for the maintenance of mitochondrial functions. Fusion helps mitigate stress by mixing the contents of partially damaged mitochondria as a form of complementation. Fission is needed to create new mitochondria, but it also contributes to quality control by enabling the removal of damaged mitochondria and can facilitate apoptosis during high levels of cellular stress.

Abstract

The placenta ages as pregnancy advances yet its continued function is required for a successful pregnancy outcome. Placental aging is a physiological phenomenon, however there are some placentas that show signs of aging earlier than others. Premature placental senescence and aging are implicated in a number of adverse pregnancy outcomes, including fetal growth restriction (FGR), preeclampsia, spontaneous preterm birth, and intrauterine fetal death. Here we discuss cellular senescence, a state of terminal proliferation arrest and how senescence is regulated. We also explore the role of physiologic placental senescence and how aberrant placental senescence alters placental function contributing to the pathophysiology of FGR, preeclampsia, spontaneous preterm labor/birth and unexplained fetal death.

Keywords: fetal growth restriction; SGA; preeclampsia; fetal death; stillbirth; membrane rupture; preterm labor; preterm birth; aging; cellular senescence; telomere; placental aging; oxidative stress; ROS; DNA damage; mTOR; PI3K, MAPK, CDK, tumor suppressor protein p53; p16; senescence-associated heterochromatin foci, SAHF; senescence-associated secretory phenotype, SASP; senescence-associated beta-galactosidase, SA-β-gal

Cellular Senescence and Aging

A key feature of aging is a progressive loss of function at the cellular, tissue and organ level, resulting in a reduced adaptability to stress and an increased vulnerability to disease and mortality[1]. In mitotic tissues, the progressive accumulation of senescent cells is thought be one of the causal factors of aging[2]. Thus, the biomarkers of cellular senescence can be used as markers of tissue aging. Such biomarkers cellular senescence have been summarized in a later section (see section "Biomarkers of senescence"). Senescent cells within tissues contribute to the aging process and disease development by altering normal cellular function, changing the behavior of neighboring cells, degrading structural components such as the extracellular matrix and accelerating the loss of tissue regeneration capacity by reducing stem and progenitor cells.[2] Elimination of senescent cells can delay aging-associated disorders in mice[3]. Cellular senescence is a state of irreversible, terminal arrest of cell proliferation (growth), triggered by a plethora of intrinsic and extrinsic stimuli or stressors. These stimuli or stressors include short or dysfunctional telomeres, DNA damage (telomeric or genomic DNA) and DNA damage response mediators, strong mitogenic signals (e.g., overexpression of oncogenic RAS, a mutant RAS-p21 protein, RAS involves transmitting signals and activating signaling cascades, including mitogen-activated protein kinase (MAPK) and PI3K/mTOR pathways), epigenomic disruption (chromatin disruption), over expression of certain oncogenes, deteriorating mitochondrial function and oxidative stress created by reactive oxygen species (ROS) (reviewed in Refs.[4-6]) (Figure 1).

The stressors that trigger senescence act by two major pathways controlled through stabilization of the tumor suppressor protein p53, and transcriptional inactivation of the cyclin-dependent kinases (CDKs). The suppression of CDKs is produced by transcriptional activation of the CDK inhibitor p21 (also termed $p21^{Cip1}$), in concert with the CDK inhibitor, p16 (also known as $p16^{INK4a}$) and pRB (retinoblastoma tumor suppressor protein) (reviewed in Refs.[7, 8]) (Figure 1). When activated, p53 inhibits cell proliferation via activation of its transcriptional target p21[8]. Both p21 and p16 maintain the protein pRB in its hypophosphorylated and active state[8, 9]. Active pRb suppress the E2F1 (a member of E2F family of transcription factors which induce gene transcriptions that are essential for cell proliferation)-dependent expression of genes that regulate progression of G1/S phase of the cell cycle, thereby irreversibly blocks the cell cycle entry[10] (see Panel 1 for cell cycle). Silencing of E2F target genes is mediated by the pRB-dependent reorganization of chromatin into distinct heterochromatin structures that accumulate in the nucleus of senescent cells termed senescence-associated heterochromatin

foci (SAHF)[9]. Interestingly, a senescent cell can re-enter the cell cycle by inhibiting p53 if the cell senescence occurred due to activation of the p53-p21 pathway, however, cells that senesce solely via the p16–pRB pathway are unable to resume proliferation even after inhibition of p53, pRB or p16[11].

Panel 1 : Cell cycle

Cell cycle or cell-proliferation cycle is a series of events that take place in a mitotic cell in order to produce two daughter cells. In eukaryotic cells, the stages of the cell cycle are divided into two major phases: interphase and the mitotic (M) phase.

Interphase: During interphase the cell grows in size and makes a copy of the cell's DNA (called DNA replication) to prepare for the cell division. The interphase is comprised of three stages: G_1 (gap 1), S (synthesis) phase, and G_2 (gap 2).

- **G_1.** In the first gap phase the cell increases in size, copies organelles, and makes the molecular building blocks it will need in later steps. The G_1 checkpoint control mechanism ensures that everything is ready for DNA synthesis.
- **S phase.** DNA synthesis occurs during this phase. It also duplicates a microtubule-organizing structure called the centrosome. The centrosomes help separate DNA during M phase.
- **G_2.** Cell continues to grow in the second gap phase, synthesises proteins and organelles. During this phase microtubules begin to reorganize to form a spindle. The G_2 checkpoint control mechanism ensures that everything is ready to enter the M phase and divide.

M phase: During the M phase, cell growth stops and cellular energy is focused on the orderly division into two daughter cells. At this stage cell separates its DNA into two sets and divides its cytoplasm, forming two new cells.

Causes of cellular senescence. A 'critically' short telomere is thought to be one factor initiating cellular senescence. Telomeres are highly conserved repetitive DNA regions, consist of tandem arrays of the hexanucleotide sequence 'TTAGGG' in the human which is typically 10-15 kb long[12], located at the end of linear chromosomes, and are essential for chromosomal stability and cell survival[13, 14]. Telomeres protect DNA ends from double-strand breaks, end-to-end fusion and degradation by forming a protective cap with a guanine-rich single-stranded telomere overhang and telomere binding protein complexes[15, 16]. Due to an inability to replicate telomeric DNA at the ends of chromosomes (known as the 'end-replication problem' of

eukaryote DNA), telomeres are progressively shortened every time a cell divides[2]. When telomeres reach a critical minimum length, their protective structure is distorted, resulting in the exposure of DNA ends and a DNA damage response, which lead to the activation of the cellular senescence pathway[5, 14, 17, 18]. This phenomenon is commonly known as 'replicative senescence'. Telomere shortening is also accelerated as a consequence of environmental stressors and insults, such as hyperglycemia, hypoxia, and oxidative stress[19-22], which lead to the oxidation of the guanosine residues. Telomere length is regulated by the enzyme telomerase, which is a specific reverse transcriptase capable of adding telomeric repeats to the ends of the chromosome.[23] Telomerase consists of a catalytic protein component, telomerase reverse transcriptase (TERT) and an RNA template component, telomerase RNA component (TERC). TERT is considered to be the rate-limiting factor in the telomerase activity[24]. The absence of a functional telomerase or loss of telomerase activity leads to a progressive telomere shortening during cell division, resulting in telomere-dependent replicative senescence and an inability to further divide when a 'critically' short telomere length is reached[18, 23, 25].

Senescence can also be induced independently of telomere length by a process termed 'premature senescence'. Premature senescence leads to premature aging and is linked to several disease process[26]. Premature senescence occurs as a consequence of progressive DNA damage and the DNA damage response, telomere uncapping and telomere dysfunction caused by extrinsic or intrinsic stressors including oxidative stress by ROS, resulting in end-to-end fusion and aggregation of telomeric DNA[27-29]. ROS stimulates senescence by inducing DNA damage, and by engaging p53-p21 and p16-pRB signal transduction cascades, either directly or indirectly[5]. Genomic damage or epigenomic perturbation, including dysfunctional telomeres and DNA double strand breaks, activates the DNA damage response. The signal transduction pathways then lead to arrest of the cell-cycle[30].

Features of cellular senescence. Senescent cells are distinct from their proliferation-competent counterparts; the former display altered characteristics, morphologically, in gene and protein expression, and in the activation of key signaling constituents[2]. Morphologically, senescence cells are enlarged, multinucleated, often double in volume and adopt a flattened or more spindle-shaped morphology, depending on the type of senescence inducer[5]. Senescent cells are resistant to apoptosis or programmed cell death through the overexpression of the anti-apoptotic Bcl-2 protein, leading to the accumulation of these cells within tissues[31].

Senescent cells display significant changes in their secretory phenotype. Senescent cells remain metabolically active, despite their terminal growth arrest, and secrete pro-inflammatory cytokines, chemokines, growth factors, and proteases, collectively termed the senescence-associated secretory phenotype (SASP)[32]. The expression of interleukin (IL)-1α, IL-1β, IL-6, IL-8 and TNF-α have been shown to increase in senescent cells[6, 33]. Increased expression of matrix metalloproteinases (enzymes that degrade extracellular matrix proteins such as collagen and elastin) is also common[34]. The senescence-associated secretory phenotype in senescent cells can induce senescence in neighboring cells[35], alter the behaviour of surrounding cells and tissue homeostasis by activating various cell-surface receptors and their signal transduction pathways, and induce tumorigenesis and malignant progression of nearby premalignant cells[6, 8, 30, 36]. Senescence-associated β-galactosidase (SA-β-gal) activity is increased in senescent cells and has been widely used as a biomarker for cellular senescence[37]. The SA-β-gal most likely derives from increased lysosomal beta-galactosidase, associated with the increased lysosomal biogenesis that occurs in senescent cells[38]. Despite its apparent specificity for senescent cells, SA-β-gal is not required for senescence[38].

Perturbation of mitochondrial homeostasis is also an important characteristic feature of cellular senescence. Aging is generally linked to a progressive mitochondrial dysfunction[39]. Mitochondrial dysfunction is characterised by increased ROS generation, impaired mitochondrial dynamics (imbalance in fission and fusion; typically more fusion, resulting in the formation of abnormally enlarged mitochondria), depolarization of the inner membrane, which stalls the mitochondrial electron transport chain, reduced ATP generation and increased AMPK activation, reduced NAD^+/NADH ratio and altered metabolism, and mitochondrial Ca^{2+} accumulation[40]. These changes in mitochondrial function can induce the activation of p53-p21 and/or p16-pRB signaling pathways that eventually lead to cellular senescence[40] (Figure 2).

Increased mTORC1 kinase activity is a common feature of senescent cells. mTORC1 is a conserved serine/threonine kinase, belonging to the phosphoinositide 3-kinase (PI3K) family that induce anabolism by regulating protein translation, nucleotide and lipid biogenesis, and inhibit the catabolic process by blocking autophagy (a process that involves fusion of acid and proteolytic enzyme containing lysosomes with autophagosomes that contain damaged organelles and misfolded proteins that is central to the cell recycling system)[41]. Persistent mTORC1 signaling in senescence cells may result from defects in sensing of amino acids and

growth factor starvation[42]. In senescent cells increased mTORC1 activity promotes protein synthesis, while inhibiting cellular proliferation[36, 43]. mTORC1 activation activates intracellular signalling cascades that regulate mitochondrial function, and apoptosis[44], while concurrently inhibiting autophagy[45], which leads to the accumulation of damaged cellular contents including misfolded proteins, as well as lipid droplets that can be seen by light microscopy as granular cytoplasmic inclusions surrounding the nucleus of senescent cells[46, 47]. Interestingly, mTORC1 inhibition by rapamycin not only delays the progression of cellular senescence but also prevents the permanent loss of proliferative capacity and allows the arrested cells to re-enter the cell cycle[36, 48-50]. Rapamycin can also prolong lifespan of various species including yeast, flies and mice[44, 51, 52], by blocking the effects of mTORC1[36, 44]. In addition, metformin, a popular hypoglycaemic agent, has recently been shown to extend longevity in worms[53] and mice[54], possibly by modulating several age-related pathways, including, mitochondrial function and AMPK activity and the nutrient-sensing mTORC1 pathway (Reviewed in Ref. [51]).

Biomarkers of senescence. The importance of senescence in aging and several age-related pathologic conditions has led to the identification of several senescence biomarkers (Table). The current methods to assess biomarkers of cell and tissue senescence have been reviewed by Bernardes et al.[55]. Expression of β-galactosidase (SA-β-gal) is known to be one of the well-characterized and simplified methods to detect senescence in vitro culture cells well as for aged tissues in vivo. The assay which measures SA-*β*-gal activity expressed by senescent cells that can be detectable at pH 6.0 by immunohistochemistry[37]. SA-β-gal is expressed specifically in senescent cells, and but not in other cell types, and is shown to increase in an age-dependent manner in human skin samples[37] and therefore, is a most widely used and reliable marker for detection of senescent cells in a variety of species and pathologic conditions[56-61].

Another important biomarker of senescence is senescence-associated heterochromatin foci (SAHF), both in vitro culture cells and in vivo. In the senescent cell nucleus, the chromatin undergoes dramatic remodelling through the formation of domains of facultative heterochromatin foci, called SAHF, [62], which can be visualised under microscopy as compacted DAPI (4,6-diamidino-2-phenylindole)-stained punctate. SAHF irreversibly silence and repress several E2F-target genes, (e.g., Cyclin A)[62], and are triggered by p16 or p53 pathways activation[63]. Transcription starting sites are absent in SAHF regions, but are enriched in transcription-silencing histone, for example, HP1, macroH2A, H3Lys9me3 (trimethylation of

lysine 9 in histone 3)[63]. Other protein complexes, which have shown to be accumulated at SAHF, include chromatin regulators HIRA, Asf1, and HMGA, and are considered as valuable biomarkers of senescence[55].

Senescence-associated secretory phenotype (SASP), which is characterized by the secretion of inflammatory signals that resembles local immune response, is a hallmark of senescence cells. The expression of inflammatory cytokines (IL-6) or chemokines (IL-8) have now been extensively used as a biomarkers for measuring senescence in cells and in tissue[64]. p16-pRB and p53-p2 are two major cellular pathways that are involved in induction of cellular senescence as described in previous section. Increased levels and/or activity of p16, p53 or p21 have been shown to be associated with cell senescence and are considered as important biomarkers of cell senescence and tissue aging[59, 65 66-68 65]. Other cellular senescence markers include, telomere shortening and dysfunction[69, 70], and activated and persistent DNA-damage response[55].

Table: Biomarkers of Senescence

Biomarkers	**Trend**	**References**
SA-β-gal	Expressed specifically in senescent cell	37, 56-61
SAHF		
H3Lyn9me3, H1, macroH2A	Positively colocalised at	71-73
HMGA, HP1	SAHF	72, 74
HIRA, Asf1		71
SASP	Increase in cell senescence	
IL-6, IL-8		64
Senescence inducers		
p16	Increase	59, 65 66
p53/p21	Increase	67, 68 65
Telomere length and DDR	Decrease	55, 69

SA-β-gal, senescence-associated β-galactosidase; SAHF, senescence- heterochromatin foci; SASP, senescence-associated secretory phenotype; IL, interleukin; DDR, DNA damage response

Cellular Senescence and Placental Aging in Pathological Pregnancies

Physiologic and pathologic placental senescence and aging

The placental syncytiotrophoblast is a multinucleated, single layer of terminally differentiated cells covering the chorionic villi. The layer is replenished by fusion of cytotrophoblasts with the overlying layer of syncytiotrophoblast, resulting in a huge syncytium with multiple nuclei. Mature (term) placental syncytiotrophoblast displays molecular markers of cellular senescence, for example, SA-β-gal, and an increased expression of the CDK inhibitors p16 and p21, and tumor suppressor p53[75]. Heterochromatin foci can be seen within the nuclei resulting from reorganisation chromatin structures[47]. Evidence of oxidative damage and aging in the syncytiotrophoblast increases as gestation advances[76] and is associated with mTOR activation and telomere shortening. Fusion of cytotrophoblasts with the syncytiotrophoblast is a physiologic process by which differentiated cytotrophoblast cells are incorporated into the syncytiotrophoblast that starts at around twelve weeks and continues until term[77]. This process is essential to achieve the rapid and extensive expansion of the placental villi, contributing to the overall growth of the placenta and constant damage repair of the chorionic villi which is accomplished through further fusion with underlying cytotrophoblasts. This process requires an endogenous human defective retroviral fusogen encoding ERVWE1 also known as syncytin-1 (syncytin-A in mice), which is expressed in all trophoblast cell lineages. Expression of ERVWE1 causes cell fusion a process that induces cellular senescence in normal and cancer cells[75]. In syncytin-A knockout mouse embryos, failure of cytotrophoblast cell fusion results in IUGR and fetal demise in mid-gestation[78]. In humans, a reduced expression of syncytin-1 has been observed in placentas associated with IUGR and preeclampsia[79].

Trophoblast senescence is a physiological phenomenon and is expected to progress gradually as pregnancy advances to term, that is with placental aging. However, premature or accelerated senescence and aging can occur as a result of placental stress that can lead to placental and clinical pathology. Premature or accelerated senescence happens when the placenta encounters stressors including, oxidative, mitochondrial or endoplasmic reticulum stress, which therefore contribute to the pathophysiology of pregnancy complications, such as preeclampsia and FGR. Low levels of stress can induce adaptive responses, including upregulation of antioxidant capacities and cell turnover by autophagy, moderate levels may interfere with stem cell behavior and reduce cell proliferation, while elevated levels of stress can cause the release of pro-inflammatory cytokines and anti-angiogenic factors, and may contribute to the

pathophysiology of preeclampsia, while chronic stress may accelerate senescence of the trophoblast[80]. The consequences of accelerated senescence in the cytotrophoblast/syncytiotrophoblast are potentially compromised placental nutrient transport that can cause compromised fetal growth, with or without preeclampsia.

Likewise, maternal decidual cells and feto-placental membranes display features of senescence as pregnancy approaches to term[47, 81]. A progressive natural physiologic senescence and aging of decidual cells and placental membranes maybe important for modulating the cell signalling pathways that are required for the onset of labour at term. Increased expression of cellular senescence signals, including p53, p21, senescence-associated secretory phenotype (IL-6 and IL-8) and SA-β-gal from both the maternal decidua and fetal membranes has been found to be associated with labor at term[81, 82], which may contribute to human parturition. Early secretion of the senescence-associated inflammatory signals (such as IL-1β, IL-6 and IL-8) due to senescence of the chorioamniotic membranes triggered by pathologic processes may promote premature membrane rupture and spontaneous preterm labor[47, 83]. It is likely that placental aging determines pregnancy duration and parturition[84], and premature aging may lead to early onset of labour.

Placental senescence in small for gestational age fetuses and neonates

Fetal growth restriction (FGR), also called small for gestational age (SGA) is defined as an estimated fetal weight below the 10th percentile for gestational age[85], affects more than 15% of pregnancies worldwide[86]. Poor placentation and placental dysfunction are known to predispose to FGR. Placental dysfunction due to the failure of trophoblast invasion and maternal spiral artery transformation, caused by ROS-mediated oxidative stress[87-89], has been reported in FGR [90]. ROS induced oxidative damage affects membrane lipids, proteins, and nucleic acids (both DNA and RNA)[89]. In genomic and mitochondrial DNA, 8-hydroxy-2'-deoxyguanosine (8-OHdG, an oxidized derivative of deoxyguanosine) is one of the predominant forms of ROS-induced oxidative DNA lesions and has therefore been widely used as a biomarker for oxidative DNA damage. The level of 8-OHdG is reported to be significantly higher in placentas associated with FGR[91-93].

Increased trophoblast senescence has been observed in FGR. FGR placentas display senescence markers, including short telomeres, telomere aggregation or dysfunction and a reduction of telomerase activity[24, 28, 94-99]. Specifically a strong association between reduction

of placental trophoblast telomere length and FGR pregnancies has been reported[24, 94, 98-100] . An absent or a decrease in telomerase activity is also observed in the placentas from FGR pregnancies[24, 94-96]. FGR placentas display upregulation of the senescence markers p21 and p16, tumor suppressor protein p53, IL-6 and a reduced expression of anti-apoptotic protein Bcl-2[27, 94]. There is also an elevated level of SAHF in FGR placenta[100]. The presence of oxidized DNA as 8-OHdG, is also increased in placental trophoblast complicated by FGR[27]. Overall, there is a strong association between reduction of telomere length in placental trophoblast and DNA damage, and FGR, suggesting that senescence in trophoblast cells may contribute to the etiology of FGR.

Preeclampsia and placental senescence

Preeclampsia is a hypertensive disorder of pregnant women, often occurs in association with FGR. Preeclampsia is the leading cause of maternal and neonatal death, and preterm birth, affecting 5-7% pregnancies worldwide[101-103]. Preeclampsia is characterised by new-onset maternal hypertension (blood pressure ≥140/90 mm Hg), diminished uteroplacental blood flow, proteinuria (≥300 mg/24 hr), and oedema[103]. An injury to the vascular endothelium is the basic pathologic event in preeclampsia[89, 104], caused by placental oxidative and endoplasmic reticulum stress[80, 105], which are known to trigger cellular senescence, and may therefore contribute to the clinical features of this pregnancy complication. Increased placental or trophoblast senescence has been demonstrated in preeclampsia in terms of senescence biomarkers, including short telomere, telomere aggregation and dysfunction and telomerase activity, senescence-associated secretory phenotype, and expression of tumor suppressor p53, and CDKs inhibitors P16 and p21. In preeclamptic placentas, telomere length and telomerase activity is decreased, and the formation of telomere (or nuclear) aggregate (SAHF) is increased compared to placentas from normotensive women[24, 28, 106]. The expression of senescence inducers p53, p21 and p16 are higher in pregnancy complicated by preeclampsia[27, 107-109]. Moreover, a high levels of pro-inflammatory cytokines (IL-1β and IL-6) profile can be demonstrated in preeclampsia[27, 110]. DNA oxidation as measured by expression of 8-OHdG in preeclamptic placenta is higher than the healthy placentas[27].

Placental senescence in spontaneous preterm labor/birth

Preterm birth is the leading cause of neonatal death and the second leading cause of infant mortality[111]. Spontaneous preterm birth may occur after the spontaneous onset of labor with or

without preterm premature rupture of the membrane (pPROM). Both term and preterm labor occur through activation of a 'common pathway' characterised by increased myometrium contractility, cervical ripening (dilatation) and decidua/chorioamniotic membrane activation and chorioamniotic membranes rupture[112, 113], and is likened to an inflammatory activation, particularly of cytokines and chemokines, in the gestational membranes[114]. In term delivery physiological signals activate the pathway, while in preterm labor several pathologic processes or conditions induce labor by activating one or more of the components of this pathway[112]. Labor promotes alterations of gene expression in placental membranes which are compatible with the localised acute inflammatory response, without evidence of histologically observable inflammation[115]. Labor is also associated with expression of senescence-associated signals in the placental chorioamniotic membranes, for example, telomere length reduction, and increased expression of p53, p21, senescence-associated secretory phenotype (IL-6 and IL-8), and SA-β-gal, mediated through the activation of the p38 MAPK pathway[82, 116]. Senescent cells may transmit inflammatory (cytokines and chemokines, the senescence-associated secretory phenotype) and senescence-promoting signals, which may cause changes in gene expression patterns in chorioamniotic membranes (overexpression of IL-8, IL-6, TLR2 (toll-like receptor 2) and SOD (superoxide dismutase)) and in amniotic fluid (IL-1α, IL-1β, IL-6, IL-8) that stimulate labor[115]. Increased levels of anti-inflammatory cytokines and chemokines, for example, TNF-α, IL-1β, IL-6, and IL-8, have been found in cervicovaginal secretions in women who deliver preterm, that are associated with early onset labor[83], early initiation of these inflammatory signals is likely to promote premature labor. Chorioamniotic membranes from spontaneous preterm labor without acute histologic chorioamnionitis (inflammation of the fetal membranes) show signs of cellular senescence, for examples, increased levels of CDKN1A (the gene that encodes p21) and SA-β-gal, and downregulated CDK and cyclins (CCNA2, CCNB1, and CCNE1) compared to preterm not-in-labor membranes[117]. Other investigators have suggested that telomeric DNA fragments released from senescent fetal cells into the amniotic fluid induce amniotic cell senescence via p38 MAPK activation and stimulate sterile inflammatory signals that promote parturition[118]. Although there is a strong association between inflammatory activation and labor (both term and preterm), whether inflammatory signals result in the induction of labor remains unclear.

Premature senescence of the intrauterine tissues, especially of the fetal membranes, triggered by senescence stimuli such as oxidative DNA damage by ROS, may contribute to spontaneous preterm labor or pPROM[119-121], possibly via the inflammatory signals (the senescence-

associated secretory phenotype). Increased expression of the biomarkers of the senescence phenotype, for example, p53, p21 and p38 MAPK were observed in the fetal membranes in preterm births with pPROM compared to spontaneous preterm and term deliveries[121]. The senescence phenotype could be induced in vitro in term fetal membranes by exposure to cigarette smoke extract. As smoke causes oxidative stress these data suggest that ROS-mediated damage to the fetal membranes may result in premature senescence in fetal membranes in pPROM[121]. Significantly shorter telomeres are also found in fetal membranes in pPROM compared to spontaneous preterm births with intact membranes, indicating that premature senescence and aging of the placental membranes may lead to pPROM[122]. Studies using a mouse model suggest that in normal mouse pregnancy progressive uterine decidual and fetal membrane senescence occur as term approaches[81], while uterine p53 deficient transgenic mice show premature and accelerated decidual senescence, with increased levels of p21, IL-8, and other cytokines and this is associated with spontaneous preterm birth[123-125]. Interestingly, an additional deletion of the p21 gene can prevent spontaneous preterm birth, indicating that p21-dependent senescence in the decidua causes preterm birth in mouse[124].

Placental senescence and aging in late gestation and fetal death

There is evidence of oxidative damage and aging in late gestational tissues[76]. It has been hypothesised that in late pregnancy, as fetal needs for nutrients and oxygen rises, if the demands exceed the placenta's ability to transfer, the placenta experiences stress that stimulates ROS generation and oxidative stress, and the resulting oxidative damage leads to aging in the placental tissue[126, 127]. The risk of fetal death increases exponentially late in pregnancy especially after 41 weeks of gestation[128, 129] suggesting that placental aging plays a key role in the clinical features of this complication. A recent study by Maiti et al. reported that placentas from unexplained intrauterine fetal death display evidence of oxidative damage and aging[76]. Increased expression of 8-OHdG (a marker of DNA oxidation) and 4-hydroxynonenal (a marker of lipid peroxidation) have been observed in fetal death associated placentas[76], compared to term placentas, expression of both these markers have also been described to increase in aging tissues[130], such as the brain in Alzheimer's disease[131, 132]. Also, a dysregulated lysosomal distribution, an increased autophagosome size with failure autophagosome-lysosomes fusion have also been noted in placentas associated with fetal death, suggesting an overall inhibition of autophagy. Placentas from late-term pregnancies show similar changes in oxidation of DNA and lipid, lysosomal distribution, and larger autophagosomes compared to

placentas from women delivered at term[76]. Increase expression of aldehyde oxidase 1 (AOX1, an enzyme that is known to involve in ROS generation[133]), is observed in placentas from both fetal death and late-term pregnancies. In vitro placental explants deprived of growth factors show similar changes in oxidation of lipid, lysosomal distribution and autophagosomes size, which can be blocked by inhibitors of AOX1 suggesting that this enzymes plays a key role in placental aging[76]. Ferrari et al. demonstrated that unexplained fetal death associated placentas exhibit shortened telomeres[134]. The authors observed an overall 2-fold reduction of telomere length in placentas from fetal death (both early and late term) with or without growth restriction compared to term live birth placentas. They also reported that the telomere length in fetal death placentas is comparable to those of pPROM, while telomeres are shorter in fetal death compared to spontaneous preterm birth[134]. Taken together, reduced telomere length, increased DNA and lipid oxidation, and inhibition of autophagy, changes that are consistent with cellular senescence and aging, indicate that placental senescence and aging is an etiological factor in fetal death.

Concluding remarks

Senescence has both beneficial and detrimental effects on gestational tissue, depending on the cell type and timing of onset. While physiological senescence in placental trophoblasts appears to be necessary for the formation of the syncytium, and growth and function of the placenta, it is likely that placental cell senescence plays a key role in pathogenesis of a number of adverse pregnancy outcomes, including FGR, preeclampsia, spontaneous preterm birth, and intrauterine fetal death. The senescence-associated secretory phenotype, especially matrix metalloproteinase that are released by the syncytiotrophoblast in early gestational tissue may be necessary for trophoblast penetration during the lacunar stage of very early placentation[47]. There is also a link between placental senescence, and the onset of labor. Spontaneous preterm labor and pPROM may be promoted by premature and accelerated senescence of placental membranes and decidua that can be induced by several endogenous and exogenous factors, such as ROS. The physiological programming of senescence may be essential in determining the timing of labor onset. In FGR the increased expression of biomarkers of DNA damage, reduction of telomere length and telomerase activity, upregulation of senescence inducing p53 and p16, and elevated levels of senescence-associated secretory phenotype and SAHF support the concept that placental senescence and aging contribute to FGR. There is also evidence of placental oxidative DNA damage, and premature senescence in late gestational tissues.

Therefore, it would appear that aging is a key factor that may affect function in the short but important life span of the placenta.

References

1. Fedarko NS. The Biology of Aging and Frailty. Clin Geriatr Med 2011;27:27-37.
2. Burton DGA. Cellular senescence, ageing and disease. Age 2009;31:1-9.
3. Baker DJ, Wijshake T, Tchkonia T, et al. Clearance of p16(Ink4a)-positive senescent cells delays ageing-associated disorders. Nature 2011;479:232-36.
4. Rodier F, Campisi J. Four faces of cellular senescence. The Journal of Cell Biology 2011;192:547-56.
5. Kuilman T, Michaloglou C, Mooi WJ, Peeper DS. The essence of senescence. Genes Dev 2010;24:2463-79.
6. Coppé J-P, Desprez P-Y, Krtolica A, Campisi J. The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. Annu Rev Pathol 2010;5:99-118.
7. Van Deursen JM. The role of senescent cells in ageing. Nature 2014;509:439-46.
8. Campisi J, D'adda Di Fagagna F. Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol 2007;8:729-40.
9. Narita M, Nuñez S, Heard E, et al. Rb-Mediated Heterochromatin Formation and Silencing of E2F Target Genes during Cellular Senescence. Cell 2003;113:703-16.
10. Gire V, Dulić V. Senescence from G2 arrest, revisited. Cell Cycle 2015;14:297-304.
11. Beauséjour CM, Krtolica A, Galimi F, et al. Reversal of human cellular senescence: roles of the p53 and p16 pathways. The EMBO Journal 2003;22:4212-22.
12. Moyzis RK, Buckingham JM, Cram LS, et al. A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. Proc Natl Acad Sci U S A 1988;85:6622-26.
13. Zhao J, Miao K, Wang H, Ding H, Wang DW. Association between Telomere Length and Type 2 Diabetes Mellitus: A Meta-Analysis. PLoS ONE 2013;8:e79993.
14. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990;345:458-60.
15. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet 2005;6:611-22.

16. O'sullivan RJ, Karlseder J. Telomeres: protecting chromosomes against genome instability. Nat Rev Mol Cell Biol 2010;11:171-81.
17. D'adda Di Fagagna F, Reaper PM, Clay-Farrace L, et al. A DNA damage checkpoint response in telomere-initiated senescence. Nature 2003;426:194-98.
18. Allsopp RC, Harley CB. Evidence for a critical telomere length in senescent human fibroblasts. Exp Cell Res 1995;219:130-6.
19. Elks CE, Scott RA. The Long and Short of Telomere Length and Diabetes. Diabetes 2014;63:65-67.
20. Saretzki G, Von Zglinicki T. Replicative aging, telomeres, and oxidative stress. Ann N Y Acad Sci 2002;959:24-29.
21. Aviv A. The Epidemiology of Human Telomeres: Faults and Promises. J Gerontol A Biol Sci Med Sci 2008;63:979-83.
22. Von Zglinicki T, Saretzki G, Döcke W, Lotze C. Mild Hyperoxia Shortens Telomeres and Inhibits Proliferation of Fibroblasts: A Model for Senescence? Exp Cell Res 1995;220:186-93.
23. Greider CW. Telomeres, telomerase and senescence. BioEssays 1990;12:363-69.
24. Biron-Shental T, Sukenik-Halevy R, Sharon Y, et al. Short telomeres may play a role in placental dysfunction in preeclampsia and intrauterine growth restriction. Am J Obstet Gynecol 2010;202:381 e1-7.
25. Greider CW. Mammalian telomere dynamics: healing, fragmentation shortening and stabilization. Curr Opin Genet Dev 1994;4:203-11.
26. Menon R, Fortunato SJ, Yu J, et al. Cigarette smoke induces oxidative stress and apoptosis in normal term fetal membranes. Placenta 2011;32:317-22.
27. Londero AP, Orsaria M, Marzinotto S, et al. Placental aging and oxidation damage in a tissue micro-array model: an immunohistochemistry study. Histochem Cell Biol 2016;146:191-204.
28. Sukenik-Halevy R, Amiel A, Kidron D, Liberman M, Ganor-Paz Y, Biron-Shental T. Telomere homeostasis in trophoblasts and in cord blood cells from pregnancies complicated with preeclampsia. American Journal of Obstetrics and Gynecology 2016;214:283.e1-83.e7.
29. Lombard DB, Schwer B, Alt FW, Mostoslavsky R. SIRT6 in DNA repair, metabolism and ageing. J Intern Med 2008;263:128-41.
30. Campisi J. Aging, Cellular Senescence, and Cancer. Annu Rev Physiol 2013;75:685-705.

31. Raffetto JD, Leverkus M, Park H-Y, Menzoian JO. Synopsis on cellular senescence and apoptosis. J Vasc Surg 2001;34:173-77.
32. Coppé J-P, Patil CK, Rodier F, et al. Senescence-Associated Secretory Phenotypes Reveal Cell-Nonautonomous Functions of Oncogenic RAS and the p53 Tumor Suppressor. PLoS Biol 2008;6:e301.
33. Minamino T, Yoshida T, Tateno K, et al. Ras induces vascular smooth muscle cell senescence and inflammation in human atherosclerosis. Circulation 2003;108:2264-9.
34. Sandeman SR, Faragher RG, Allen MC, Liu C, Lloyd AW. Does MMP-2 expression and secretion change with increasing serial passage of keratocytes in culture? Mech Ageing Dev 2001;122:157-67.
35. Nelson G, Wordsworth J, Wang C, et al. A senescent cell bystander effect: senescence-induced senescence. Aging Cell 2012;11:345-9.
36. Xu S, Cai Y, Wei Y. mTOR Signaling from Cellular Senescence to Organismal Aging. Aging Dis 2014;5:263-73.
37. Dimri GP, Lee X, Basile G, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci U S A 1995;92:9363-7.
38. Lee BY, Han JA, Im JS, et al. Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. Aging Cell 2006;5:187-95.
39. Wang Y, Hekimi S. Mitochondrial dysfunction and longevity in animals: Untangling the knot. Science 2015;350:1204-07.
40. Ziegler DV, Wiley CD, Velarde MC. Mitochondrial effectors of cellular senescence: beyond the free radical theory of aging. Aging Cell 2015;14:1-7.
41. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell 2012;149:274-93.
42. Carroll B, Nelson G, Rabanal-Ruiz Y, et al. Persistent mTORC1 signaling in cell senescence results from defects in amino acid and growth factor sensing. The Journal of Cell Biology 2017;10.1083/jcb.201610113.
43. Demidenko ZN, Blagosklonny MV. Growth stimulation leads to cellular senescence when the cell cycle is blocked. Cell Cycle 2008;7:3355-61.
44. Wei Y, Zhang Y-J, Cai Y, Xu M-H. The role of mitochondria in mTOR-regulated longevity. Biological Reviews 2015;90:167-81.
45. Kim J, Kundu M, Viollet B, Guan K-L. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol 2011;13:132-41.

46. Ogrodnik M, Salmonowicz H, Brown R, et al. Dynamic JUNQ inclusion bodies are asymmetrically inherited in mammalian cell lines through the asymmetric partitioning of vimentin. Proceedings of the National Academy of Sciences 2014;111:8049-54.
47. Cox LS, Redman C. The role of cellular senescence in ageing of the placenta. Placenta 2017;52:139-45.
48. Demidenko ZN, Zubova SG, Bukreeva EI, Pospelov VA, Pospelova TV, Blagosklonny MV. Rapamycin decelerates cellular senescence. Cell Cycle 2009;8:1888-95.
49. Kolesnichenko M, Hong L, Liao R, Vogt PK, Sun P. Attenuation of TORC1 signaling delays replicative and oncogenic RAS-induced senescence. Cell Cycle 2012;11:2391-401.
50. Pospelova TV, Leontieva OV, Bykova TV, Zubova SG, Pospelov VA, Blagosklonny MV. Suppression of replicative senescence by rapamycin in rodent embryonic cells. Cell Cycle 2012;11:2402-7.
51. Romero R, Erez O, Huttemann M, et al. Metformin, the aspirin of the 21st century: its role in gestational diabetes mellitus, prevention of preeclampsia and cancer, and the promotion of longevity. Am J Obstet Gynecol 2017;217:282-302.
52. Harrison DE, Strong R, Sharp ZD, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 2009;460:392-95.
53. Cabreiro F, Au C, Leung KY, et al. Metformin retards aging in C. elegans by altering microbial folate and methionine metabolism. Cell 2013;153:228-39.
54. Martin-Montalvo A, Mercken EM, Mitchell SJ, et al. Metformin improves healthspan and lifespan in mice. Nat Commun 2013;4:2192.
55. Bernardes De Jesus B, Blasco MA. Assessing Cell and Organ Senescence Biomarkers. Circ Res 2012;111:97-109.
56. Melk A, Schmidt BM, Takeuchi O, Sawitzki B, Rayner DC, Halloran PF. Expression of p16INK4a and other cell cycle regulator and senescence associated genes in aging human kidney. Kidney Int 2004;65:510-20.
57. Mishima K, Handa JT, Aotaki-Keen A, Lutty GA, Morse LS, Hjelmeland LM. Senescence-associated beta-galactosidase histochemistry for the primate eye. Invest Ophthalmol Vis Sci 1999;40:1590-3.
58. Castro P, Giri D, Lamb D, Ittmann M. Cellular senescence in the pathogenesis of benign prostatic hyperplasia. Prostate 2003;55:30-8.

59. Krishnamurthy J, Torrice C, Ramsey MR, et al. Ink4a/Arf expression is a biomarker of aging. J Clin Investig 2004;114:1299-307.
60. Sun LQ, Lee DW, Zhang Q, et al. Growth retardation and premature aging phenotypes in mice with disruption of the SNF2-like gene, PASG. Genes Dev 2004;18:1035-46.
61. Cao L, Li W, Kim S, Brodie SG, Deng CX. Senescence, aging, and malignant transformation mediated by p53 in mice lacking the Brca1 full-length isoform. Genes Dev 2003;17:201-13.
62. Zhang R, Chen W, Adams PD. Molecular dissection of formation of senescence-associated heterochromatin foci. Mol Cell Biol 2007;27:2343-58.
63. Funayama R, Ishikawa F. Cellular senescence and chromatin structure. Chromosoma 2007;116:431-40.
64. Coppe JP, Patil CK, Rodier F, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 2008;6:2853-68.
65. Serrano M, Lin AW, Mccurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 1997;88:593-602.
66. Coppe JP, Rodier F, Patil CK, Freund A, Desprez PY, Campisi J. Tumor suppressor and aging biomarker p16(INK4a) induces cellular senescence without the associated inflammatory secretory phenotype. J Biol Chem 2011;286:36396-403.
67. Chen Z, Trotman LC, Shaffer D, et al. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature 2005;436:725-30.
68. Tyner SD, Venkatachalam S, Choi J, et al. p53 mutant mice that display early ageing-associated phenotypes. Nature 2002;415:45-53.
69. Bernadotte A, Mikhelson VM, Spivak IM. Markers of cellular senescence. Telomere shortening as a marker of cellular senescence. Aging 2016;8:3-11.
70. Von Zglinicki T, Martin-Ruiz CM. Telomeres as biomarkers for ageing and age-related diseases. Curr Mol Med 2005;5:197-203.
71. Zhang R, Poustovoitov MV, Ye X, et al. Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Dev Cell 2005;8:19-30.
72. Narita M, Nunez S, Heard E, et al. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 2003;113:703-16.

73. Rogakou EP, Sekeri-Pataryas KE. Histone variants of H2A and H3 families are regulated during in vitro aging in the same manner as during differentiation. Exp Gerontol 1999;34:741-54.
74. Narita M, Narita M, Krizhanovsky V, et al. A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. Cell 2006;126:503-14.
75. Chuprin A, Gal H, Biron-Shental T, et al. Cell fusion induced by ERVWE1 or measles virus causes cellular senescence. Genes Dev 2013;27:2356-66.
76. Maiti K, Sultana Z, Aitken RJ, et al. Evidence that fetal death is associated with placental aging. Am J Obstet Gynecol 2017;10.1016/j.ajog.2017.06.015.
77. Goldman-Wohl D, Yagel S. United we stand not dividing: The syncytiotrophoblast and cell senescence. Placenta 2014;35:341-44.
78. Dupressoir A, Vernochet C, Bawa O, et al. Syncytin-A knockout mice demonstrate the critical role in placentation of a fusogenic, endogenous retrovirus-derived, envelope gene. Proceedings of the National Academy of Sciences 2009;106:12127-32.
79. Ruebner M, Strissel PL, Ekici AB, et al. Reduced Syncytin-1 Expression Levels in Placental Syndromes Correlates with Epigenetic Hypermethylation of the ERVW-1 Promoter Region. PLoS One 2013;8:e56145.
80. Burton GJ, Yung HW, Murray AJ. Mitochondrial – Endoplasmic reticulum interactions in the trophoblast: Stress and senescence. Placenta 2017;52:146-55.
81. Bonney EA, Krebs K, Saade G, et al. Differential senescence in feto-maternal tissues during mouse pregnancy. Placenta 2016;43:26-34.
82. Menon R, Behnia F, Polettini J, Saade GR, Campisi J, Velarde M. Placental membrane aging and HMGB1 signaling associated with human parturition. Aging 2016;8:216-30.
83. Bowen JM, Chamley L, Keelan JA, Mitchell MD. Cytokines of the Placenta and Extra-placental Membranes: Roles and Regulation During Human Pregnancy and Parturition. Placenta 2002;23:257-73.
84. Menon R, Bonney EA, Condon J, Mesiano S, Taylor RN. Novel concepts on pregnancy clocks and alarms: redundancy and synergy in human parturition. Hum Reprod Update 2016;22:535-60.
85. The American College of Obstetricians and Gynecologists (ACOG). ACOG Practice bulletin no. 134: fetal growth restriction. Obstet Gynecol 2013;121:1122-33.

86. Lu L, Kingdom J, Burton GJ, Cindrova-Davies T. Placental Stem Villus Arterial Remodeling Associated with Reduced Hydrogen Sulfide Synthesis Contributes to Human Fetal Growth Restriction. Am J Pathol 2017;187:908-20.
87. Huppertz B. Placental origins of preeclampsia challenging the current hypothesis. Hypertension 2008;51:970-75.
88. Hung TH, Skepper JN, Charnock-Jones DS, Burton GJ. Hypoxia-reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia. Circ Res 2002;90:1274-81.
89. Sultana Z, Maiti K, Aitken J, Morris J, Dedman L, Smith R. Oxidative stress, placental ageing-related pathologies and adverse pregnancy outcomes. Am J Reprod Immunol 2017;77:e12653.
90. Lyall F, Bulmer JN, Duffie E, Cousins F, Theriault A, Robson SC. Human trophoblast invasion and spiral artery transformation: the role of PECAM-1 in normal pregnancy, preeclampsia, and fetal growth restriction. Am J Pathol 2001;158:1713-21.
91. Takagi Y, Nikaido T, Toki T, et al. Levels of oxidative stress and redox-related molecules in the placenta in preeclampsia and fetal growth restriction. Virchows Archiv 2004;444:49-55.
92. Fujimaki A, Watanabe K, Mori T, Kimura C, Shinohara K, Wakatsuki A. Placental oxidative DNA damage and its repair in preeclamptic women with fetal growth restriction. Placenta 2011;32:367-72.
93. Kimura C, Watanabe K, Iwasaki A, et al. The severity of hypoxic changes and oxidative DNA damage in the placenta of early-onset preeclamptic women and fetal growth restriction. J Matern Fetal Neonatal Med 2013;26:491-96.
94. Davy P, Nagata M, Bullard P, Fogelson NS, Allsopp R. Fetal growth restriction is associated with accelerated telomere shortening and increased expression of cell senescence markers in the placenta. Placenta 2009;30:539-42.
95. Kudo T, Izutsu T, Sato T. Telomerase activity and apoptosis as indicators of ageing in placenta with and without intrauterine growth retardation. Placenta 2000;21:493-500.
96. Kim S-Y, Lee S-P, Lee J-S, Yoon S-J, Jun G, Hwang Y-J. Telomerase and Apoptosis in the Placental Trophoblasts of Growth Discordant Twins. Yonsei Med J 2006;47:698-705.

97. Biron-Shental T, Kidron D, Sukenik-Halevy R, et al. TERC telomerase subunit gene copy number in placentas from pregnancies complicated with intrauterine growth restriction. Early Human Development 2011;87:73-75.
98. Toutain J, Prochazkova-Carlotti M, Cappellen D, et al. Reduced Placental Telomere Length during Pregnancies Complicated by Intrauterine Growth Restriction. PLoS One 2013;8:e54013.
99. Biron-Shental T, Sukenik Halevy R, Goldberg-Bittman L, Kidron D, Fejgin MD, Amiel A. Telomeres are shorter in placental trophoblasts of pregnancies complicated with intrauterine growth restriction (IUGR). Early Hum Dev 2010;86:451-56.
100. Biron-Shental T, Sukenik-Halevy R, Sharon Y, Laish I, Fejgin MD, Amiel A. Telomere shortening in intra uterine growth restriction placentas. Early Hum Dev 2014;90:465-9.
101. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science 2005;308:1592-94.
102. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet 2005;365:785-99.
103. Myatt L, Cui X. Oxidative stress in the placenta. Histochem Cell Biol 2004;122:369-82.
104. Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, Mclaughlin MK. Preeclampsia: an endothelial cell disorder. Am J Obstet Gynecol 1989;161:1200-04.
105. Hubel CA, Roberts JM, Taylor RN, Musci TJ, Rogers GM, Mclaughlin MK. Lipid peroxidation in pregnancy: new perspectives on preeclampsia. Am J Obstet Gynecol 1989;161:1025-34.
106. Sukenik-Halevy R, Fejgin M, Kidron D, et al. Telomere aggregate formation in placenta specimens of pregnancies complicated with pre-eclampsia. Cancer Genet Cytogenet 2009;195:27-30.
107. Sharp AN, Heazell AE, Baczyk D, et al. Preeclampsia is associated with alterations in the p53-pathway in villous trophoblast. PLoS One 2014;9:e87621.
108. Gao Q, Zhu X, Chen J, Mao C, Zhang L, Xu Z. Upregulation of P53 promoted G1 arrest and apoptosis in human umbilical cord vein endothelial cells from preeclampsia. J Hypertens 2016;34:1380-8.
109. Nuzzo AM, Giuffrida D, Masturzo B, et al. Altered expression of G1/S phase cell cycle regulators in placental mesenchymal stromal cells derived from preeclamptic pregnancies with fetal-placental compromise. Cell Cycle 2017;16:200-12.

110. Campos-Cañas J, Romo-Palafox I, Albani-Campanario M, Hernández-Guerrero C. An imbalance in the production of proinflammatory and anti-inflammatory cytokines is observed in whole blood cultures of preeclamptic women in comparison with healthy pregnant women. Hypertens Pregnancy 2014;33:236-49.
111. Liu L, Johnson HL, Cousens S, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet 2012;379:2151-61.
112. Romero R, Dey SK, Fisher SJ. Preterm Labor: One Syndrome, Many Causes. Science 2014;345:760-65.
113. Romero R, Gomez R, Mazor M, Ghezzi F, Yoon BH. The preterm labor syndrome. In: Elder MG, Romero R, Lamont RF, eds. *Preterm Labor*. New York: Churchill Livingstone, 1997.
114. Keelan JA, Marvin KW, Sato TA, Coleman M, Mccowan LM, Mitchell MD. Cytokine abundance in placental tissues: evidence of inflammatory activation in gestational membranes with term and preterm parturition. Am J Obstet Gynecol 1999;181:1530-36.
115. Haddad R, Tromp G, Kuivaniemi H, et al. Human spontaneous labor without histologic chorioamnionitis is characterized by an acute inflammation gene expression signature. Am J Obstet Gynecol 2006;195:394.e1-24.
116. Behnia F, Taylor BD, Woodson M, et al. Chorioamniotic membrane senescence: a signal for parturition? Am J Obstet Gynecol 2015;213:359.e1-16.
117. Gomez-Lopez N, Romero R, Plazyo O, et al. Preterm labor in the absence of acute histologic chorioamnionitis is characterized by cellular senescence of the chorioamniotic membranes. Am J Obstet Gynecol 2017;10.1016/j.ajog.2017.08.008.
118. Polettini J, Behnia F, Taylor BD, Saade GR, Taylor RN, Menon R. Telomere Fragment Induced Amnion Cell Senescence: A Contributor to Parturition? PLoS One 2015;10:e0137188.
119. Polettini J, Dutta EH, Behnia F, Saade GR, Torloni MR, Menon R. Aging of intrauterine tissues in spontaneous preterm birth and preterm premature rupture of the membranes: A systematic review of the literature. Placenta 2015;36:969-73.
120. Menon R. Oxidative stress damage as a detrimental factor in preterm birth pathology. Front Immunol 2014;5:567.

121. Menon R, Boldogh I, Hawkins HK, et al. Histological evidence of oxidative stress and premature senescence in preterm premature rupture of the human fetal membranes recapitulated in vitro. Am J Pathol 2014;184:1740-51.
122. Menon R, Yu J, Basanta-Henry P, et al. Short Fetal Leukocyte Telomere Length and Preterm Prelabor Rupture of the Membranes. PLOS ONE 2012;7:e31136.
123. Cha J, Hirota Y, Dey SK. Sensing senescence in preterm birth. Cell Cycle 2012;11:205-6.
124. Hirota Y, Cha J, Yoshie M, Daikoku T, Dey SK. Heightened uterine mammalian target of rapamycin complex 1 (mTORC1) signaling provokes preterm birth in mice. Proceedings of the National Academy of Sciences 2011;108:18073-78.
125. Hirota Y, Daikoku T, Tranguch S, Xie H, Bradshaw HB, Dey SK. Uterine-specific p53 deficiency confers premature uterine senescence and promotes preterm birth in mice. J Clin Invest 2010;120:803-15.
126. Burton GJ, Jauniaux E. Placental Oxidative Stress: From Miscarriage to Preeclampsia. J Soc Gynecol Investig 2004;11:342-52.
127. Smith R, Maiti K, Aitken R. Unexplained antepartum stillbirth: A consequence of placental aging? Placenta 2013;34:310-13.
128. Yudkin PL, Wood L, Redman CWG. RISK OF UNEXPLAINED STILLBIRTH AT DIFFERENT GESTATIONAL AGES. Lancet 1987;329:1192-94.
129. Smith GCS. Life-table analysis of the risk of perinatal death at term and post term in singleton pregnancies. American Journal of Obstetrics and Gynecology 2001;184:489-96.
130. Hirano T, Yamaguchi R, Asami S, Iwamoto N, Kasai H. 8-hydroxyguanine levels in nuclear DNA and its repair activity in rat organs associated with age. J Gerontol A Biol Sci Med Sci 1996;51:B303-7.
131. Lovell MA, Markesbery WR. Oxidative DNA damage in mild cognitive impairment and late-stage Alzheimer's disease. Nucleic Acids Res 2007;35:7497-504.
132. Markesbery W, Lovell M. Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer’s disease. Neurobiol Aging 1998;19:33-36.
133. Kundu TK, Velayutham M, Zweier JL. Aldehyde Oxidase Functions as a Superoxide Generating NADH Oxidase: An Important Redox Regulated Pathway of Cellular Oxygen Radical Formation. Biochemistry 2012;51:2930-39.

134. Ferrari F, Facchinetti F, Saade G, Menon R. Placental telomere shortening in stillbirth: a sign of premature senescence? The Journal of Maternal-Fetal & Neonatal Medicine 2016;29:1283-88.

List of Figures

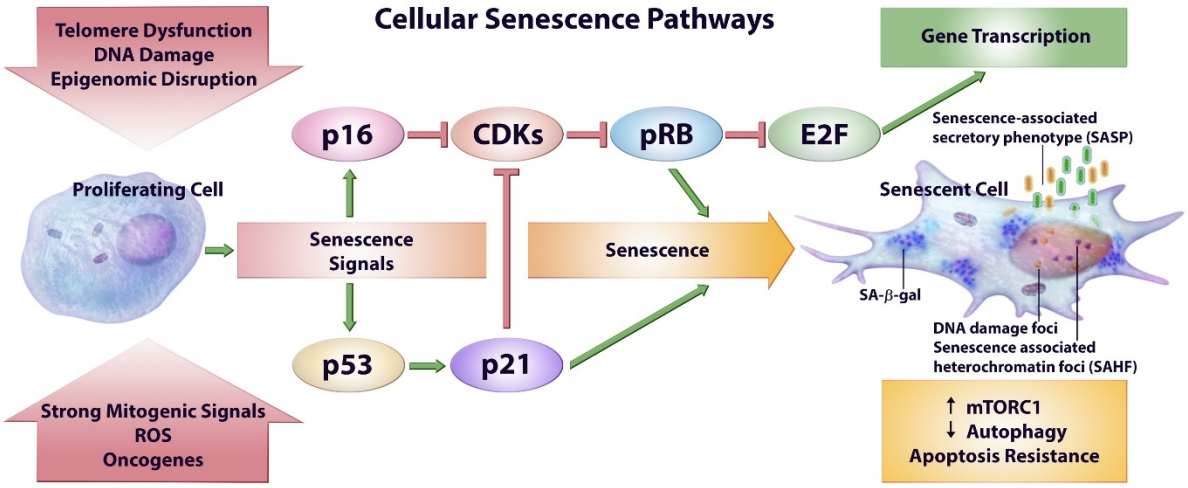

Figure 1 **An overview of Cellular senescence**. Telomere-dependent replicative senescence and stress-induced premature senescence act through the modulation of proteins p53 and Rb. Senescence stimuli, such as DNA damage, strong mitogenic signals, overexpression of oncogenes, epigenomic disruption, telomere dysfunction and ROS engage in cell signaling cascades that cause activation of one or both of the pathways that regulate cell senescence, the p53-p21 and p16-pRb pathways. Activation of p53 induces the expression of a CDK inhibitor, p21. Senescence stimuli, which involve the p16–pRB pathway upregulate the expression of another CDK inhibitor, p16. Both p21 and p16 suppress the phosphorylation and inactivation of pRB, and hereby maintain its hypophosphorylated and active state. Active pRB halts cell cycle progression by inhibiting gene transcription via downregulating transcription factor E2F. Senescent cells remain metabolically active, despite their terminal growth arrest, and secrete pro-inflammatory cytokines, chemokines, growth factors, and proteases, collectively termed the senescence-associated secretory phenotype.

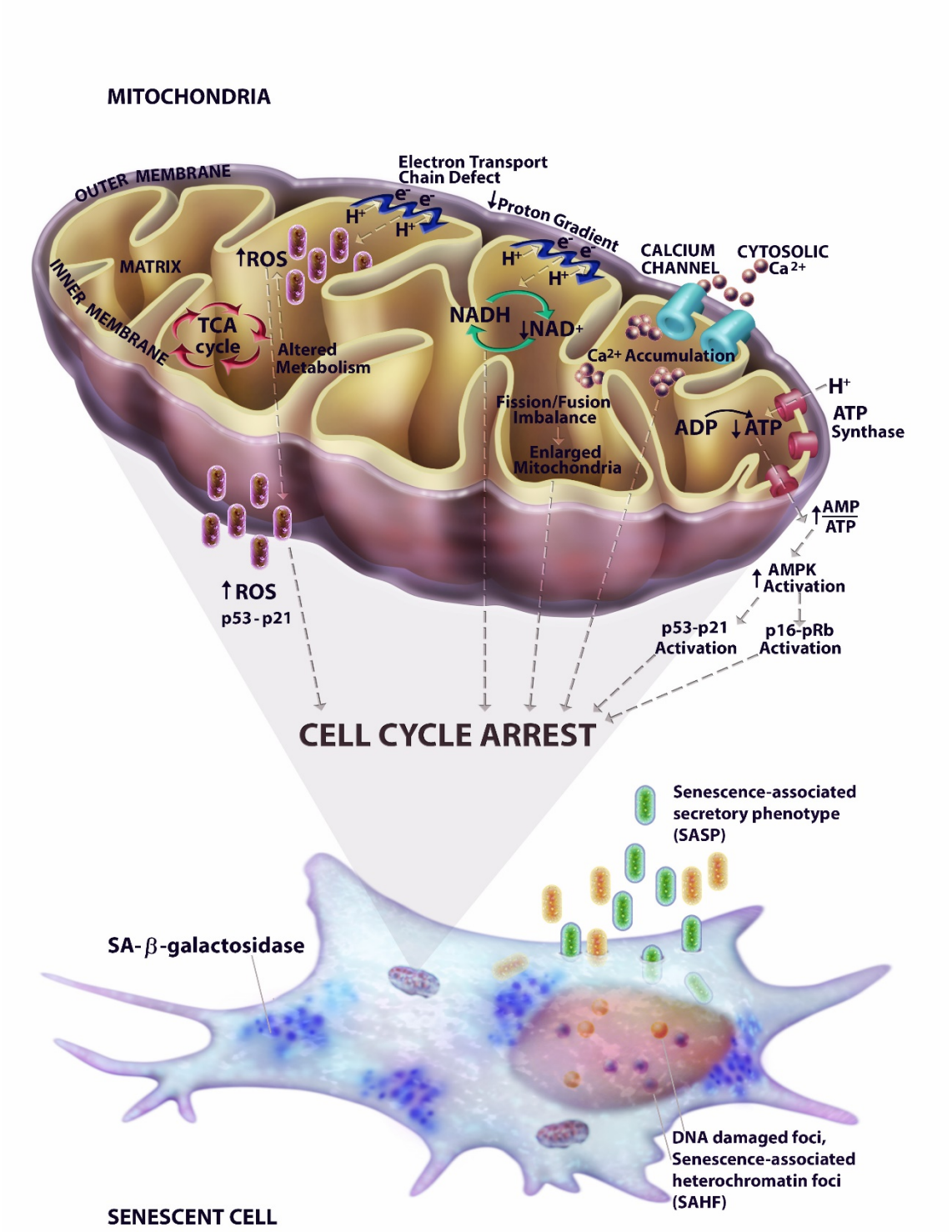

Figure 2 **Perturbation of mitochondrial homeostasis.** Changes in mitochondrial function trigger cellular senescence via activation of p53-p21 and/or p16-pRB signal transduction cascades. Figure adapted from Ziegler et al.[40].