

The role of cellular senescence in ageing of the placenta

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Abstract

Aberrant placental ageing is implicated in a high percentage of birth complications, stillbirths and neonatal deaths. Understanding how this complex organ is established and maintained for the 9-10 months of pregnancy and then how and why it undergoes the physiological changes that result in labour at term is therefore of enormous clinical importance. In this review, we assess the evidence that placental ageing results from cellular senescence, a state of terminal proliferation arrest accompanied by characteristic morphological and metabolic changes including a shift to a pro-inflammatory phenotype. We discuss how senescence both contributes to placental formation during cytotrophoblast fusion, and to the changes necessary for labour onset, such as cervical remodelling and increased sterile inflammatory signalling. Based on evidence from human clinical studies and experimental interventions in mice, we assess possible biochemical pathways that may drive senescence, and speculate on how aberrant senescence in the placenta may contribute to pre-eclampsia, pre-term birth and stillbirth.

Key words: placenta; senescence; syncytiotrophoblast; decidua; inflammation; IUGR; pre-eclampsia; stillbirth; senescence-associated secretory phenotype (SASP); aging

Abbreviations: FIS (fusion-induced senescence); IUGR (intra-uterine growth restriction); OIS (oncogene-induced senescence); PE (preeclampsia); PTB (pre-term birth); RS (replicative senescence); SA- β -gal (senescence-associated beta galactosidase); SAHF (senescence-

associated heterchromatin foci); SASP (senescence-associated secretory phenotype); SIPS (stress-induced premature senescence).

Highlights

- Cellular senescence is a state of proliferation arrest with altered morphology and acquisition of a pro-inflammatory secretome, the SASP
- Fusion of cytotrophoblasts to form the syncytiotrophoblast (STB) promotes STB senescence
- Secretion of the SASP by the STB may be required for tissue remodelling to support placental function
- Senescence of maternal decidual cells may promote changes leading to birth

Introduction

The burden of placental pathologies

Placental pathology causes major pregnancy disorders, partially encompassed in the concept of the Great Placental Syndromes [1]. The original concept was limited to disorders of deep placentation and included preeclampsia, fetal growth restriction without preeclampsia, preterm labour, preterm premature rupture of membranes, late spontaneous abortion, and abruptio placentae. However most cases of preeclampsia are of late onset where placentation is not an issue [2] although the disorder is also secondary to placental pathology. While gestational diabetes is not part of this group because the placental pathology is secondary to the maternal condition, not its cause, it imposes stresses on the placenta.

The global burden of these conditions is heavy. Pre-eclampsia alone accounts for one sixth of direct maternal deaths, about 60,000 per annum [3]. About 15 million babies are born preterm each year and the rate appears to be increasing [4]. About three quarters are spontaneous and the rest induced largely because of preeclampsia [5]. There are 2.6 million stillbirths/year [6]. Even those that previously would have been considered to be unexplained are increasingly found to be associated with important placental pathology.

Understanding the underlying placental pathology of these conditions is therefore key in developing strategies to limit or prevent them. One issue that is re-emerging is the importance of post-term gestation which not only is implicated in elevated rates of stillbirth but associated with increased preeclampsia rates [2]. In this context placental ageing may be a key component in determining the outcome of pregnancy. In this review we assess the current state of understanding of the role of cellular senescence in the placenta, including how senescence may normally contribute both to placental formation and the normal onset of labour, and how aberrant senescence can lead either to PTB or to post-term stillbirth. We further consider how treatments targeting senescence within the placenta may lead to novel therapeutic strategies for intrauterine growth restriction and preeclampsia.

Features of cell senescence

Cellular senescence is a state of essentially irreversible cell cycle arrest resulting from high levels of the cyclin kinase inhibitors p21^{CDKN1} and/or p16^{INK4/6/CDKN2} as well as tumour suppressors p53 and/or pRb (retinoblastoma tumour suppressor protein) (reviewed in [7]). Senescent cells are generally larger than proliferating cells of the same lineage, and show a flattened morphology with marked actin stress fibres [8]. Increased mTORC1 kinase activity in senescent cells promotes protein synthesis through activation of ribosomal biogenesis [9] and relief of translational inhibition [10], though without the expected downstream response of cellular proliferation normally observed on mTORC activation in the non-senescent state. Autophagy inhibition by active mTORC signalling [11] results in accumulation of cellular debris that can be visualised by light microscopy as granular cytoplasmic inclusions in JunQ bodies close to the nucleus of senescent cells [12]; lipid droplets are also often seen (Figure 1). A widely used biomarker of senescence is high lysosomal activity characterised by pH-sensitive senescence-associated beta galactosidase (SA-β-gal, [13]), which identifies senescent cells *in vitro* and *in vivo*. Senescent cells can be mono- or multi-nucleate, and often show enlarged nuclei with aberrant distribution of heterochromatin and prominent nucleoli. Lamin B loss is commonly observed [14], and chronic DNA damage is thought to persist, since senescent cells stain strongly for γH2AX, a marker of unrepaired DNA double strand breaks (reviewed in [15]).

As well as their characteristic morphology, senescent cells markedly change gene expression patterns, with overexpression of anti-apoptotic Bcl-2 leading to resistance to apoptosis [16]. In parallel, high NFκB activity results in the expression of proinflammatory cytokines and chemokines which contribute to the senescence-associated secretory phenotype, or SASP [17]. The composition of the SASP varies according to cell lineage [18], but generally includes canonical markers IL-6 and TNF-α; secretion of metalloproteases such as MMP3 and MMP9 is also common. Very recent studies in primary oral fibroblasts demonstrated that upregulation of COX2 and PGE2 may trigger SASP production [19]. The SASP may also be responsible for induction of 'bystander senescence' in surrounding cells [20].

Acute senescence is important in development and wound healing

Cellular senescence can arise in response both to physiological and pathological stimuli (Table 1). Developmental senescence has been described as a physiological process important for tissue remodelling in the early embryo; cells staining positive for senescence markers p21^{CDKN1} and SA-β-gal can be detected at limb buds and in developing organ structures in mice [21, 22], and it is thought that the secreted SASP factors recruit NK cells for immunological clearance of senescent cells [23] that is necessary for remodelling. In the axolotl, senescence is critical for limb regeneration after amputation [24], suggesting a role in acute wound healing. Senescent cells also play an important though complex role in mammalian wound healing, promoting fibrosis in the lung but preventing liver fibrosis (reviewed in [25]).

In addition to wound healing, senescence is thought to be a potent tumour suppressor mechanism that halts proliferation of cells with acute oncogene activation, and permits immunological removal of these potentially neoplastic cells [26]. This type of senescence is known as oncogene-induced senescence (OIS). Similarly, high levels of DNA damage, converging on p53, p21 and p16, can lead to cell cycle arrest that then proceeds into senescence (reviewed in [25]).

Senescence contributes to ageing

Acute senescence is therefore beneficial to multicellular organisms, either in development, wound healing or in preventing hyperplasia of cells with genomic damage or activated oncogenes. However, replicative exhaustion from telomere shortening [27], and/or chronic macromolecular damage, such as persistent DNA lesions, lipid peroxidation, and protein carbonylation that accumulate over long periods of time can result in sustained triggering of the senescence pathway and accumulation of senescent cells with organismal age [25]. Such accumulation leads to loss of tissue repair capacity accompanied by a damaging state of chronic inflammation that is thought to contribute to frailty and many of the diseases associated with old age, including cardiovascular disease, cancer and dementia [23]. Indeed, senescent cells can be identified post-mortem at sites of hemodynamic stress, such as arterial junctions (especially in aortic aneurysms [28]). They have recently been implicated in neurodegeneration with age [29], including in Alzheimer's and Parkinson's disease [30, 31]. Inhibition of mTORC1 using rapamycin leads to lifespan extension in mice [32], and improves cognitive function in both AD and PD mouse models [33, 34]; rapamycin acts at least in part by suppressing the SASP [35] suggesting that chronic inflammation may be a key feature in the pathologies resulting from senescent cell accumulation. Recent studies in mice show tissue rejuvenation when senescent cells are removed using genetic techniques or by inducing senescent cell apoptosis [36-39], highlighting the detrimental nature of persistent senescent cells. Hence senescent cells are strongly associated with loss of tissue and organ function with increasing age.

Formation of the syncytiotrophoblast may induce senescence

Cell fusion, an essential physiological process to establish and expand the syncytiotrophoblast (STB), has recently been recognised to be a further trigger of cell senescence ([40], see also Table 1). Senescence as a response to fusion may have evolved to halt the proliferation of cells infected with fusogenic viruses (e.g. the measles virus) so it is of note that cytotrophoblast fusion requires a retroviral fusogen hERVWE1 (also known as syncytin 1 in humans and syncytin A in mice), that is now encoded within the mammalian genome. Fusion is necessary to achieve the rapid and extensive 13-fold expansion of this tissue that occurs between 12 weeks and term [41] to give the massive single cell syncytium (12m² and at least 10¹⁰ nuclei at term). Microvillous syncytial sprouts break off into maternal blood and constant repair is required – which is accomplished through further fusion with underlying

1 cytotrophoblasts. Hence fusion continues as an ongoing process throughout pregnancy and
2 is not simply an initiating event in STB formation. Failure of cytotrophoblast cell fusion in
3 mouse embryos, null for syncytin-A, results in IUGR and fetal death in mid-pregnancy, with
4 deficient vascularisation [42], while in humans, syncytin-1 expression is down-regulated in
5 placental syndromes including IUGR and PE [43, 44]. In cases where fusion occurs but is
6 limited, the placenta forms but the STB is small and hypofunctional, resulting in intrauterine
7 growth restriction and pre-term birth. For example, syncytium formation is defective in
8 Down's syndrome [45], potentially because excess superoxide dismutase (encoded on
9 chromosome 21) prevents the build up of ROS that would otherwise direct cytotrophoblast
10 differentiation towards fusion and STB formation [46].

11
12 The STB shows features characteristic of senescent cells including the biomarker SA- β -gal,
13 together with high expression of the cyclin kinases inhibitors p16 and p21, and p53 [40].
14 These prevent cell cycle progression and cell division, which could be disastrous for a
15 syncytium of this size and complexity. Within the syncytium, newly recruited nuclei show
16 some residual DNA synthesis, older syncytial nuclei (present in syncytial knots) lack DNA
17 synthesis [47] but instead show chromatin rearrangement to form heterochromatic foci
18 reminiscent of SAHFs found in replicative senescence and oncogene-induced senescence
19 (OIS) [48]. Consistent with its other senescent phenotypes, the STB also secretes
20 immunomodulatory and tissue remodelling factors including matrix metalloproteases [49].
21 The key features of the senescent STB are shown schematically in Figure 2. As well as
22 trophoblast senescence on STB formation, it has also been reported that natural killer cells
23 are induced to undergo senescence in response to soluble HA-G secreted by trophoblasts,
24 and that this promotes remodelling of the maternal spiral arteries in early pregnancy [50].

25
26 Apart from its barrier and transfer functions the STB is also a major source of newly
27 synthesised proteins and lipid. For example, pregnancy maintenance requires production of
28 hCG by the STB [51]. To achieve a high level of protein synthesis, extensive ribosome
29 biogenesis is required together with enhanced rates of translation, accomplished through
30 elevated mTORC1 signalling. Notably, elevated mTORC signalling without proliferation is
31 also a key feature of other modes of senescence.

Hence STB senescence is a normal physiological phenomenon. As would be expected, it progresses as pregnancy advances, that is with ageing. The evidence that this occurs is more complete in animal than in human pregnancies (summarised by [52]). There is increasing evidence that pathology when physiological senescence is accelerated it leads to placental and clinical pathology. There are two groups of outcomes. First, if ageing affects the STB its transfer functions may be compromised leading to fetal growth restriction, with or without pre-eclampsia. Secondly, if ageing affects the chorioamnion it may promote labour, either normally or prematurely.

Placental pathology and senescence

Poor placentation predisposes to early-onset pre-eclampsia, which is strongly associated with IUGR, or can cause normotensive IUGR, with similar placental pathology. Both are associated with a burden of underlying placental oxidative and endoplasmic reticulum stress [52, 53], which are known to accelerate cellular senescence, and may therefore contribute to the clinical features of these major placental syndromes. Increased placental or trophoblast senescence has been demonstrated in preeclampsia or normotensive IUGR, in terms of telomere shortening, aggregation or other measures of telomere dysfunction [54-57]. Overall, there is a definite trend of decreasing placental telomere length with IUGR [58], suggesting that telomere-attrition dependent senescence in decidual cells may contribute to early onset of labour (see Figure 3).

The frequency of SAHFs correlates with IUGR [56] and increases on exposure to senescence-inducing agents such as ROS [59]. By analysing the patterns of differential DNA methylation in the placenta it is possible to estimate its chronological age (normal placentas) or detect accelerated aging. In this way accelerated ageing has been found in placentas associated with early but not late onset pre-eclampsia [60].

Microarray analysis of human placentas of various gestational ages has demonstrated that expression of other markers of senescence, particularly p21, p53, APE1 and IL-6, is increased in placental syndromes including PE and IUGR [61], while short telomeres in trophoblasts are associated with placental syndromes such as PE [57]. Proteomics comparisons show upregulation of

senescence factors (e.g. annexins) in PE placentas compared with those from normal pregnancies [62, 63], again highlighting the link between senescence and placental syndromes

Late gestation stillbirth and the ageing placenta

It is possible that ageing of the placenta also plays a role in late gestation stillbirth. After 40 weeks of gestation, the death rate for fetuses rises [64]. Some deaths may be associated with overt or occult IUGR [65]. A recent study of unexplained stillbirths reveals placental senescence in terms of significantly shortened telomeres [66]. It is likely that post-term, fetal demands for oxygen and nutrients outstrip the placenta's ability to supply, and that an inherent build-up of ROS can lead to deterioration of the placenta including marked senescence [67].

Senescence of the decidua and amniochorion, in relation to labour.

Fetal tissues comprise only a part of the placenta, as the decidua is formed from differentiated maternal stromal fibroblasts that have undergone decidualisation. Such cells are responsible for the secretion of an extracellular matrix rich in fibronectin and laminin, but they also promote enhanced vascular permeability. As pregnancy comes to term, decidual cells show many features of senescence including secretion of SASP factors such as IL-6. As with STB senescence, decidual senescence is associated with increased mTORC1 signalling, with preterm birth associating with elevated mTOR in mice and humans [68]. (see Figure 3). A recent analysis of senescence markers in membranes from term labours shows a clear upregulation of factors associated with senescence together with the proinflammatory SASP [69, 70]. A gradual process of decidual senescence may be critical for driving the cellular and tissue changes that contribute to labour onset at term. If ageing of the placenta normally determines pregnancy duration, then the natural corollary is that premature placental ageing will lead to pre-term labour onset, as has recently be reviewed in detail [71]. It has been argued that increasing release of cell free placental DNA as part of a senescence associated stress reaction may trigger preterm labour [72]. Part of the cell free placental DNA may include telomere fragments which, with released HMGB1, could act as DAMPs (damage associated molecular pattern (DAMP) markers to promote sterile inflammation contributing to the onset of labour [71].

Transgenic mice with uterine p53 deletion show accelerated decidual senescence, with elevated p21, IL-8 and various CXCL cytokines that together contribute to the SASP [73]. This deletion correlates with preterm birth, which can be rescued by additional deletion of the p21 gene [73], strongly supporting the hypothesis that p21-dependent senescence causes PTB.

As telomere attrition is a known driver of replicative and DNA-damage induced senescence, many studies of placental pathology have analysed telomere length. Of particular note are trisomy 21 pregnancies, where short placental telomeres and early decidual senescence correlate with intrauterine growth restriction [74]. There is as yet no definitive association between telomere length and other specific pathologies, perhaps because damage at only one telomere can trigger senescence while most assays measure global telomere length across cell populations e.g. in clinical biopsies.

Senescence as a potential inducer of labour

As the pregnancy comes to term, elevated sterile inflammatory signals (the SASP) secreted from both the senescent maternal decidua and fetal membranes are likely to contribute to onset of labour. In particular, elevation of prostaglandins PGF₂ and PGE₂ acting through the COX2 pathway are thought to be important in signalling labour onset [75]; this inflammatory signalling is exacerbated by infection (e.g. [68]). Breakdown of collagen networks is required for remodelling of the cervix and extracellular matrix to permit birth, and it is highly likely that the metalloproteases in the SASP contribute to this. Early activation of this sterile inflammation is likely to trigger premature labour, and this has indeed been observed in the clinic where elevated IL-1 β , IL-6 and IL-8 levels are associated with early onset labour (reviewed in [76]) as is metalloprotease MMP8 [77]. As in other types of senescence, the pro-labour SASP depends on NF κ B-mediated transcription [78]. Moreover, a high pro-inflammatory, low anti-inflammatory cytokine profile can be demonstrated in preeclampsia [79], suggestive of a role for the SASP in the damaging pathologies of PE.

Suppression of the pro-inflammatory SASP by inhibiting mTORC1 or p38MAPK signalling is pharmacologically possible, at least in cell culture [35, 80, 81]. Notably, in transgenic mouse

models of premature placental senescence, single dose administration of rapamycin can rescue the premature birth phenotype [73]. In mice with acute LPS-induced inflammation, the humanised antibody tocilizumab, which targets the IL-6 receptor, prevented preterm birth [82]; however, in humans, the safety data are inconclusive [83]. The role of inflammation is also equivocal, given that indomethacin has been used for many years for the treatment of preterm labour without clear clinical benefit [84]. It should also be cautioned that high levels of inflammatory mediators are not simply indicative of placental cell senescence and can arise from other stressors (hypoxia) or liberation of danger signals especially microvesicles or necrotic debris [85], or from pathology in other anatomical sites - for instance, activated maternal endothelium. Hence, while it is pharmacologically possible to suppress the SASP of senescent cells in cell culture, we caution that experimental data are still far too scant to justify use in human pregnancy.

Conclusions

Placental senescence raises several important questions that need to be addressed experimentally. While fusion-induced senescence appears to be required for syncytiotrophoblast formation, it is likely that senescence of both fetal tissues and the maternal decidua play at least a part in determining timing of labour onset (Table 2). In terms of the SASP, some factors may be beneficial and some detrimental to maintaining pregnancy, and it may depend on which cells are producing the SASP. There are marked differences between cell types as to what factors are secreted in the SASP. For example MMPs secreted by the STB in the first trimester may be necessary for trophoblast penetration during the lacunar stage of very early placentation, while those produced by the decidua (and probably the fetal membranes) in late stage pregnancy may contribute to cervical remodelling in preparation for labour. Non-physiological causes of senescence such as ROS resulting from maternal smoking, exposure to environmental pollutants, or general poor perfusion (e.g. from failure to fully remodel spiral arteries) are likely to trigger stress-induced senescence earlier than the normal programme of senescence; it is notable that these factors are all linked and are associated with pre-term birth. However, what determines the rate of accumulation of senescent cells and expression of SASP factors in uncomplicated pregnancies is as yet unknown.

Ageing is one of the highest risk factors known for most adult human diseases, such as cancer, diabetes, and metabolic syndrome [86]. It would seem that placental ageing is likewise a high risk factor for a variety of conditions that affect the placenta at the end of its short life span. This perception offers opportunities for rethinking preventive strategies and understanding between constitutional and environmental factors that adversely affect pregnancy outcome.

Acknowledgements

LSC is grateful to the Glenn Foundation for Medical Research for an award for Research in Biological Mechanisms of Aging, and to the BBSRC (grant number [BB/M006727/1]).

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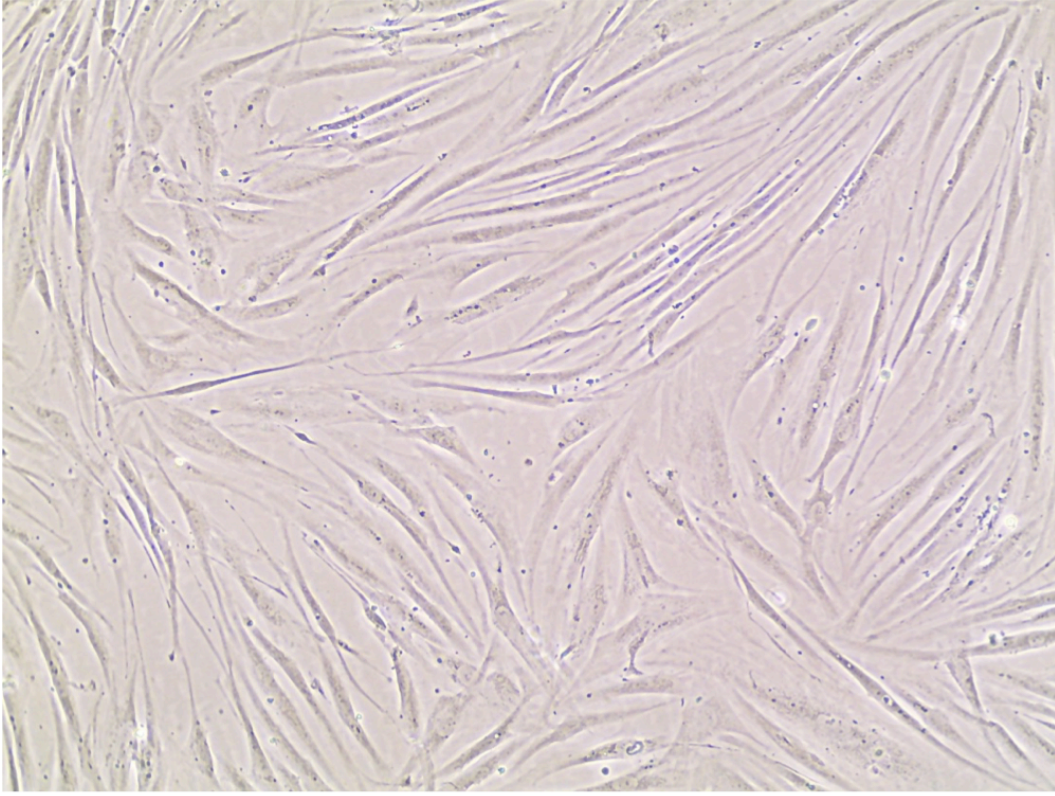
Figure legends

Figure 1. Morphology of senescent cells. (A) Proliferating human skin fibroblasts in culture, with characteristic spindle morphology. (B) Cells from the same population, grown in culture until they have undergone ~90 population doublings and reached replicative senescence. Note the massive increase in cell area, prominent nucleoli, multiple nuclei in some cells, uneven cell margins, and granular inclusions in the juxta-nuclear Q bodies (JunQ) as well as lipid droplets.

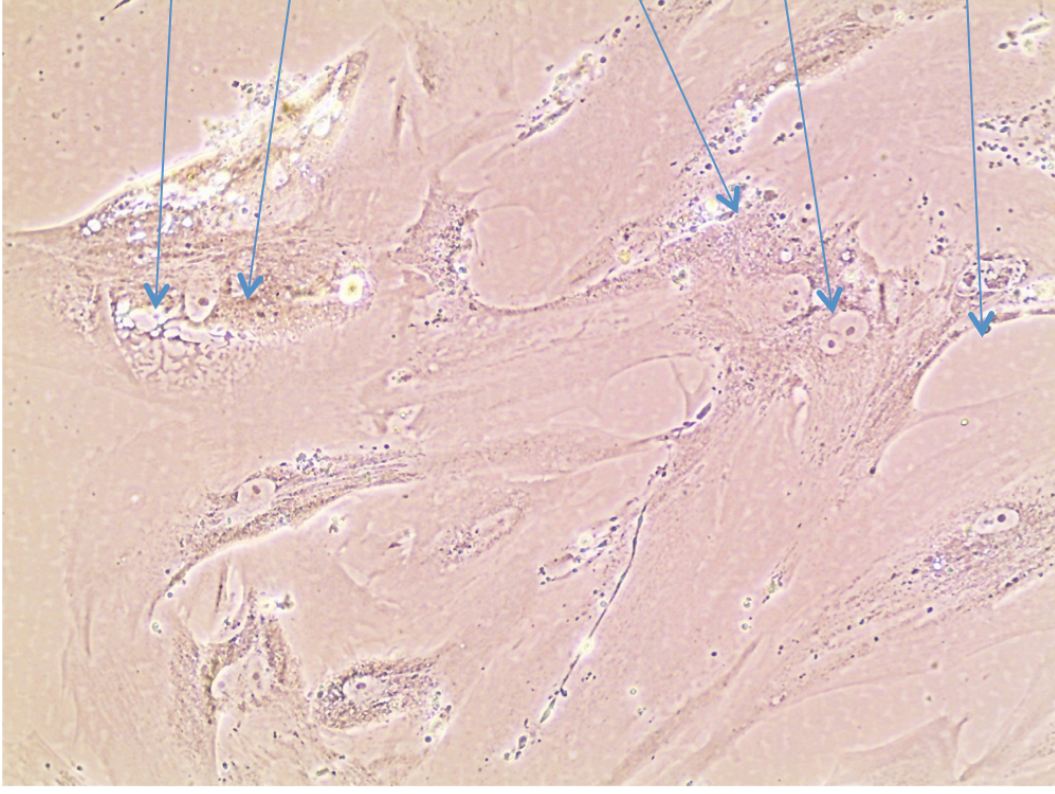
Figure 2. Features of senescence in the syncytiotrophoblast. Cartoon image showing fusion of proliferation-competent cytotrophoblasts to form the non-dividing syncytiotrophoblast. Fusion is mediated by the retroviral syncytin-1 protein, resulting in an enormous syncytium with many nuclei; such fusion is thought to drive senescence. Chromatin reorganisation within the nuclei results in formation of heterochromatin foci. The role of the STB is both to act as a barrier and a transfer surface, and to provide proteins such as chorionic gonadotrophin. High levels of protein synthesis may be supported by active mTORC1 signalling, while cell division is blocked by elevated cyclin kinase inhibitors p21 and p16. Telomere length within the STB is somewhat controversial though short STB telomeres are associated with IUGR and PTB. The STB secretes factors that influence surrounding tissues, including matrix metalloproteases. In later stages of gestation, secretion of pro-inflammatory mediators may help to promote labour. These factors make up the SASP (senescence-associated secretory phenotype).

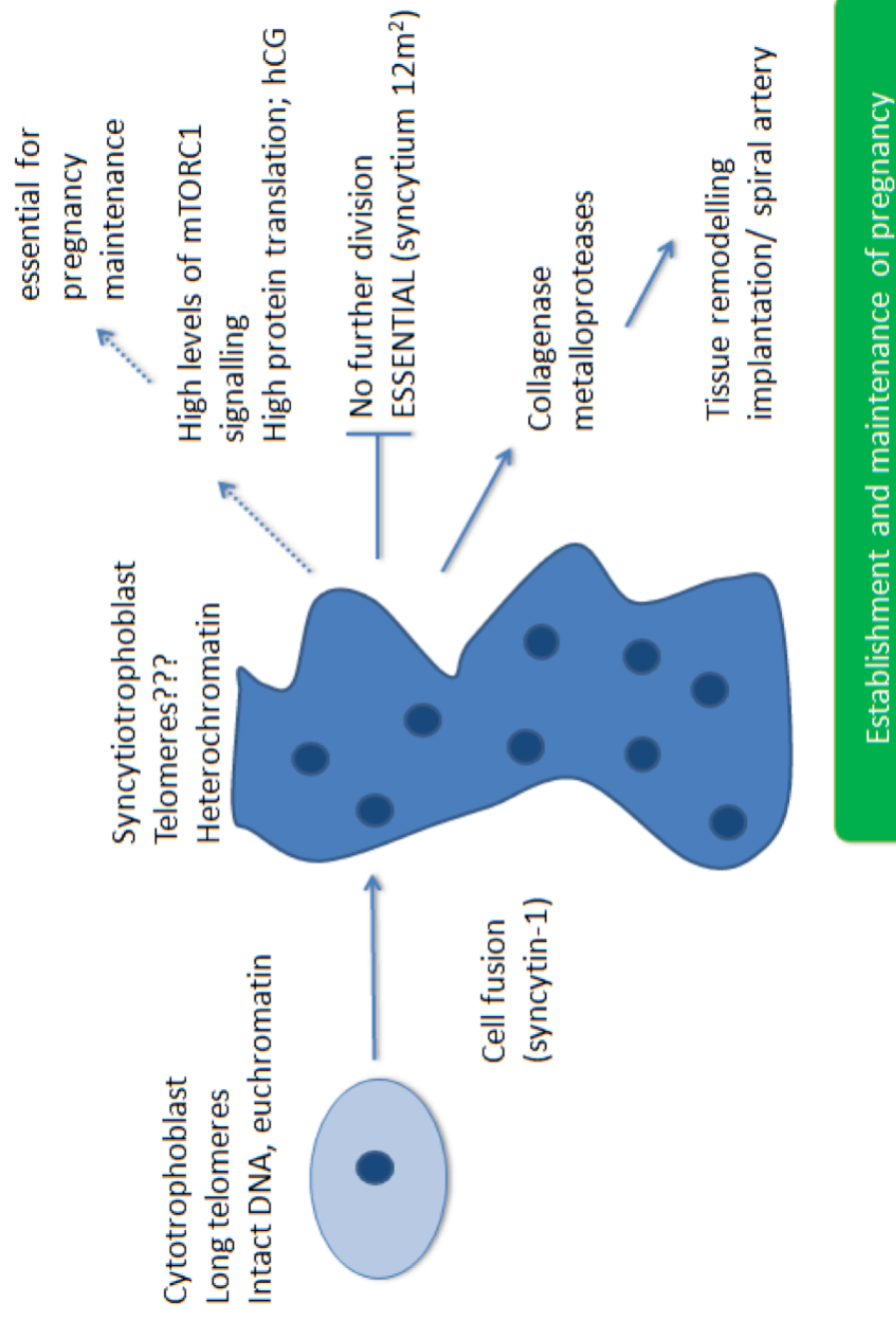
Figure 3. Biochemical pathways contributing to senescence in pregnancy. Senescence may be induced prematurely in the placenta by various factors (red box) including ROS and exposure to agents that cause DNA damage; trisomy 21 also alters ROS balance and is associated with short placental telomeres. Premature onset of senescence leads to early production of the SASP (senescence-associated secretory phenotype), which includes activation of the cyclooxygenase pathways through COX2 and prostaglandins, as well as secretion of pro-inflammatory cytokines and chemokines such as IL-6 and TNF α .

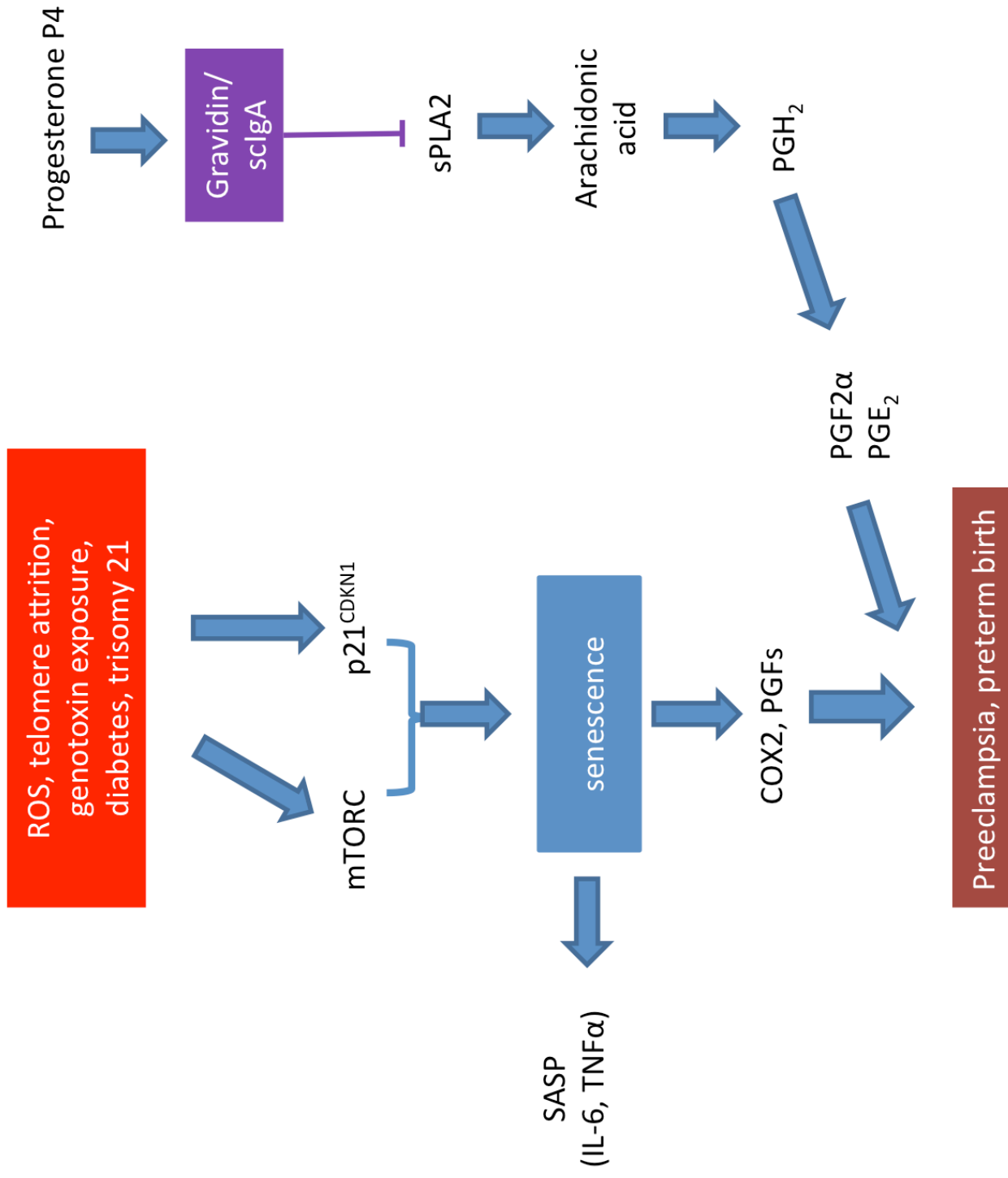
A proliferating



B senescent

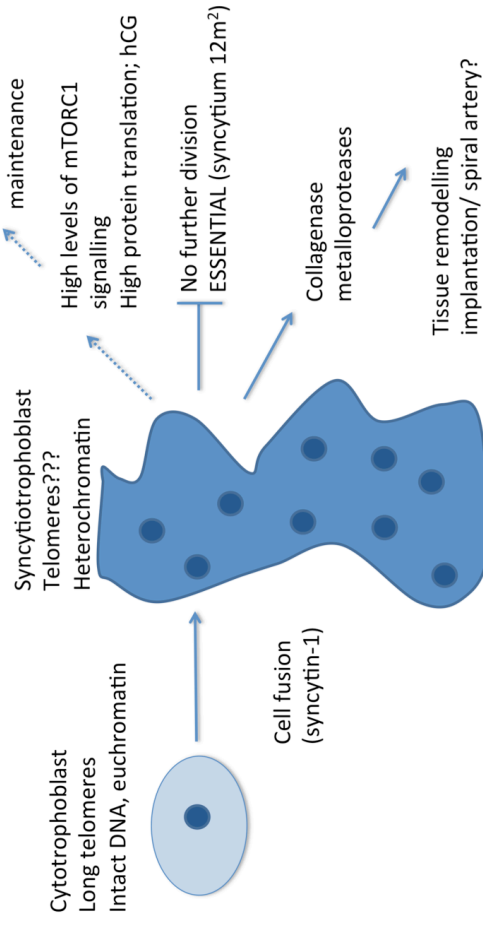






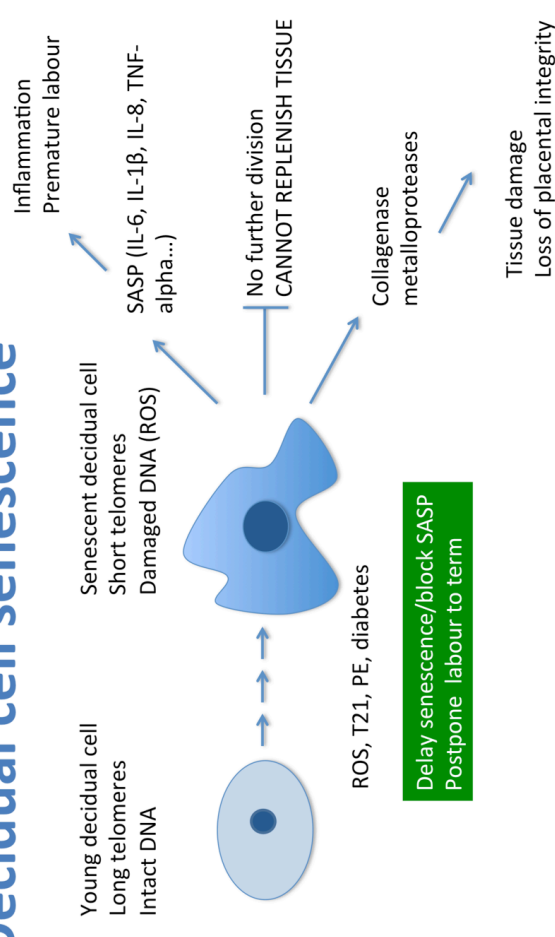
Cox and Redman Figure 3

STB senescence



Establishment and maintenance of pregnancy

Decidual cell senescence



Pre-eclampsia, pre-term birth, stillbirth