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REVIEW

Cellular senescence and developmental defects

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Cellular senescence is a distinct state that is frequently induced in response to ageing and stress. Yet studies have also uncovered beneficial functions in development, repair and regeneration. Current opinion therefore suggests that timely and controlled induction of senescence can be beneficial, while misregulation of the senescence program, either through mis-timed activation, or chronic accumulation of senescent cells, contributes to many disease states and the ageing process. Whether atypical activation of senescence plays a role in the pathogenesis of developmental defects has been relatively underexplored. Here, we discuss three recent studies that implicate ectopic senescence in neurodevelopmental defects, with possible causative roles for senescence in these birth defects. In addition, we highlight how the examination of senescence in other birth defects is warranted, and speculate that aberrantly activated senescence may play a much broader role in developmental defects than currently appreciated.

Introduction

Senescence mechanisms and functions

Cellular senescence is a state of permanent cell cycle arrest, entered into by cells at the end of their replicative lifespan. Additionally, senescence can be induced in response to damage signals such as oncogenes, irradiation and chemotherapy. In response to such inducers, senescent cells arrest from proliferation, which is largely mediated by the activation of cell-cycle inhibitors such as p53, p21, p16^{Ink4a} or p19^{Arf} [1,2]. Senescent cells also exhibit many characteristic phenotypic features (often dependent on the inducer) including enlarged cell size, cytoplasmic chromatin fragments, heterochromatin foci, and enlarged and often

dysfunctional lysosomes [3]. This latter feature also helps with the identification of senescent cells, which exhibit increased acidic lysosomal beta-galactosidase activity, which can be detected by staining known as senescence-associated beta-galactosidase (SA β -gal) [4]. However, senescent cells are not dead, and in many cases exhibit increased resistance to apoptotic stimuli. They remain metabolically active, interacting extensively with their microenvironment by producing many secreted factors including proteins (chemokines, cytokines, extracellular matrix proteins and remodelers) and exosomes, in what is collectively known as the senescence-associated secretory phenotype (SASP) [5].

Abbreviations

AER, apical ectodermal ridge; ASD, autism-spectrum disorder; BP, basal progenitors; CdLS, Cornelia de Lange syndrome; CGs, cardiac glycosides; DDR, DNA-damage response; DEGs, differentially expressed genes; DQ, dasatinib + quercetin; DS, Down syndrome; E, embryonic day; EEC, ectrodactyly ectodermal dysplasia cleft lip/palate; EMT, epithelial-mesenchymal transition; HDACi, histone deacetylase inhibitor; iPSCs, induced pluripotent stem cells; miRNA, microRNA; NECs, neuroepithelial cells; NK, natural killer; NPCs, neural progenitor cells; NT, neural tube; NTD, neural tube defects; Ras/MAPK, Ras/Map kinase; RG cells, radial glial cells; SASP, senescence-associated secretory phenotype; Sa β -gal, senescence-associated beta-galactosidase; VPA, valproic acid.

The functions of this secretome are complex and varied, and include stimulating the recruitment of immune cells, that remove the senescent cells. This was initially shown in the tumour setting, where senescent cancer cells were removed by macrophages and natural killer (NK) cells [6,7]. In addition, the SASP exerts widespread additional effects on neighbouring cells, depending on the context, including promoting proliferation, angiogenesis, EMT, cell plasticity and stemness and even paracrine senescence [8–10].

The biological roles of senescent cells are complex, and probably still not entirely understood.

On the one hand, beneficial functions have been described for senescent cells in various contexts. They protect from cancer, preventing oncogene-expressing cells from aberrant proliferation, with senescence thereby acting as a tumour suppressive mechanism [11,12]. Additional beneficial roles have been identified in a number of non-pathological conditions. Senescent cells are present transiently during embryonic development in many species, where they are thought to contribute to proper tissue development and patterning, before being removed by macrophages [13–16]. Developing tissues where senescence has been demonstrated include the apical ectodermal ridge (AER) of the limb; hindbrain roofplate, mesonephros (kidney) and the endolymphatic sac. Interestingly, here it appears that a primary mediator of senescence in the embryo is p21, without expression of age-associated markers p16^{Ink4a} and p19^{Arf}. Recent reviews elaborate on the roles of senescence during development in greater detail [17–19]. Additionally, senescent cells transiently appear and contribute to tissue repair and regeneration, for example in the skin, heart and in axolotl appendage regeneration [20–23]. In such cases, the SASP plays critical roles in communicating with neighbouring cells. In each of these scenarios, the timely induction and subsequent removal of the senescent cells by immune system seems to be key.

On the other hand however, in many ageing tissues, the number of senescent cells increases over time. Here, the accumulation of non-proliferating senescent cells, and prolonged SASP exposure from these, has detrimental effects, and is thought to impair tissue function and to actively contribute to the ageing process [24–27]. Although not fully understood, this is thought to partly result from an impairment in their timely removal [28]. In addition, the mis-timed induction or chronic accumulation of senescent cells is a contributing factor to many age-associated diseases. This includes but is not limited to osteoarthritis, liver fibrosis, chronic-obstructive pulmonary disease, Alzheimer's and Parkinson's disease, and many more

[27,29–31]. In such diseases, the persistence of non-proliferating and inflammatory senescent cells is thought to exacerbate disease symptoms. For both ageing and associated diseases, mounting evidence shows that genetic- and drug-mediated removal of senescent cells can be beneficial for many pathologies, with loss of senescence leading to improved tissue function or an amelioration of disease symptoms. Such results have led to a dramatic increase in the identification of drugs with suggested capacity to target senescent cells, broadly referred to as senotherapeutics. These generally act by either killing them (senolytics) or interfering with the SASP and senescent state (senomorphics) [26,27,32–34].

Therefore, it can be considered that senescent cells are transient, arrested, secretory cells, induced by diverse signals and which should ultimately be removed by the immune system. However, when the controlled process of induction or removal is perturbed, this population of cells persists and accumulates, exerting their effects beyond the destined time, contributing to ageing, cancer and other disease states.

With regards to senescence in disease, in most cases it appears that senescent cells are not a primary cause of the disease, but act as a contributing factor that exacerbates the pathology. During embryonic development, senescence is found in many tissues that are prone to being affected by developmental defects and disorders such as the neural tube, limb and ear. This raises the intriguing question of whether aberrant induction, or mis-timed activation of senescence in the embryo might also contribute to embryonic diseases and developmental defects. While this concept has received relatively little attention, recent studies are beginning to uncover how aberrantly induced senescence may not only contribute to, but may actually play a causative role in developmental defects (Fig. 1). Such studies further our understanding of how ectopic induction of senescence can contribute to disease, and highlight how the study of senescence in additional developmental defects is warranted.

Senescence and developmental defects

Senescence and fetal valproate spectrum disorder

One recent study identified a role for senescence in developmental disorders induced in response to the teratogen Valproic Acid (VPA), a medication used to treat a variety of disorders including epilepsy, bipolar disorder, migraine and other conditions [35] (Fig. 1A).

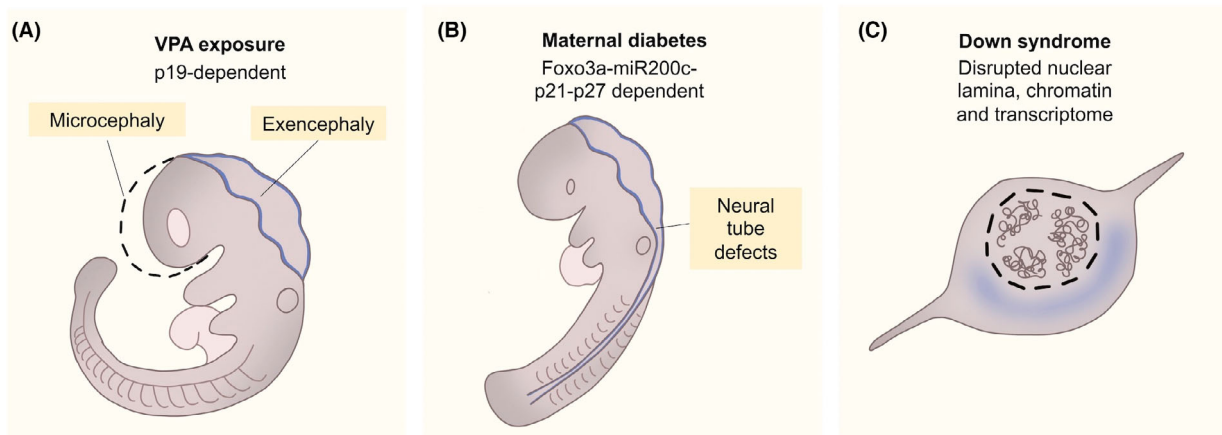


Fig. 1. Three models of ectopic senescence leading to neurodevelopmental defects. (A) Rhinn *et al.* [35] reported ectopic senescence in NECs of the neural tube of E9.5 embryos following *in-utero* VPA exposure. The phenotype included exencephaly and forebrain microcephaly and relied on p19^{Arf} expression. (B) Xu *et al.* [46] showed ectopic senescence along the neural tube in E8.5 embryos due to maternal diabetes. Embryos displayed neural tube defects, and senescence was triggered by Foxo3a-miR200c-p21-p27 pathway. (C) Meharena *et al.* [50] reported that NPCs differentiated from iPSCs of Down syndrome patients displayed disrupted nuclear lamina, altered chromatin state and transcriptome.

It emerged in the 1960s that children born to pregnant mothers taking this drug had a significantly higher incidence of major developmental defects [36–38]. Most common among these was an increased incidence of spina bifida, a situation where the posterior neural tube fails to close properly during development, resulting in the spinal cord projecting outside the newborn, and necessitating the need for complex surgery and treatment. Interestingly, when modelled in mice this also results in exencephaly, a lethal condition where the anterior neural tube fails to close, resulting in the brain developing outside of the head [39,40].

During subsequent years, it was documented in human patients that exposure to VPA *in utero* caused many additional birth defects including higher incidence in microcephaly, digit patterning defects, heart defects and more, and with a higher risk associated with higher doses [36]. Furthermore, over time it became clear that infants who were born following embryonic exposure to VPA additionally had significant cognitive and behavioural problems, including a significantly higher risk for autism-spectrum disorder (ASD). However, what wasn't initially clear was that the cognitive and behavioural defects could occur independently of congenital defects, resulting in the former going unnoticed for a longer period of time. It is now clear that the risk of cognitive defects and ASD-like problems are far more frequent than physical deformation, occurring in approx. 30–40% of exposed infants, but with these figures also being variable depending on

the timing of exposure and the dose in question [36–38,41,42].

Valproic acid is an HDAC-inhibitor (HDACi), and is used as an anti-cancer treatment, where it can induce senescence. In particular, studies showed that it causes activation of p21 or de-repression of the *Cdkn2a* gene, leading in particular to induction of p19^{Arf} (p14^{Arf} in human) expression [43,44]. Based on this knowledge, a recent study investigated whether VPA might cause developmental disorders through the aberrant or mistimed induction of cellular senescence [35].

First, they established a mouse model of VPA-induced defects, exposing the embryos to high concentrations of VPA around embryonic day (E) 8.5, just prior to the stages of neural tube closure. Importantly, the mice developed multiple features associated with VPA, including exencephaly, microcephaly, decreased size and a gross malformation in the somites, all consistent with the congenital defects found in humans. Next, the authors examined for aberrant induction of senescence, staining whole embryos with SA β -gal. Surprisingly, there was a pronounced induction of senescence, but only in the developing brain, and not in other malformed tissues. This correlated with increased senescence-associated gene expression including activation of p21, p19^{Arf} and p16^{Ink4a}, and SASP genes.

Looking more closely, it was identified that senescence was induced at embryonic day (E) 9.5 in the neuroepithelial cells (NECs), the common embryonic precursor of all cell types in the adult brain. During

development, NECs transition to radial glial (RG) cells. Radial glial cells are early neural progenitor cells (NPCs) (also called apical progenitors). Then, through highly coordinated symmetric and asymmetric divisions, they give rise to the various cell types of the neural tissue, including neurons and microglia [45]. Here, the effect of senescence following VPA exposure was a block in the normal differentiation program of the NECs, and the brains of these embryos had fewer apical and basal progenitors (BPs), and a decrease in neurogenesis. This suggested VPA-induced senescence blocks neural differentiation during development.

Critically, the authors also examined whether VPA also induced senescence in human neural development, using human cerebral organoids grown from induced pluripotent stem cells (iPSCs) cells. Importantly, early cerebral organoids model the corresponding stages of development examined in mice. Interestingly, human organoids treated with VPA also exhibited senescence in NECs, also resulting in impaired neural differentiation.

Finally, a key question was to determine whether senescence played a causative role in the phenotypes. Here, mice deficient in each of the key senescence genes *p21*, *p19^{Arf}* and *p16^{Ink4a}* were treated with VPA and examined for the associated phenotypes. Surprisingly, while *p21*- and *p16^{Ink4a}*-deficient mice had no reduction in the severity of phenotypes, *p19^{Arf}*-deficient mice had a decreased incidence of senescence and microcephaly, which rescued the neurogenesis defects in the NECs. Conversely, genetic misexpression of *p19^{Arf}* in the NECs was sufficient to induce senescence and microcephaly. Finally, RNA-seq analysis on *p19^{Arf}*-deficient embryos supported the rescue phenotype and suggested links with control of tRNA function as a possible factor.

Importantly, this study identified a major role for *p19^{Arf}*-mediated senescence in VPA-induced neurodevelopmental defects, in particular by disrupting NEC differentiation and neurogenesis, and demonstrating that a high induction of senescence can result in microcephaly.

Senescence and diabetes-induced neural tube defects

Another study uncovered a causative role for senescence in developmental birth defects induced by maternal diabetes [46] (Fig. 1B). For as yet unknown reasons, maternal diabetes is a major risk factor for neural tube defects (NTD). As the number of diabetic women of child-bearing age is significant, there is a critical need to understand this association with

developmental defects [47]. Previous studies had identified links with diabetic embryopathy and senescence mediators, including DNA damage, p53-induction and oxidative stress [46]. To explore a potential link with senescence and diabetes-induced developmental defects, the authors used a mouse model of streptozotocin treatment to induce diabetes, which results in the induction of exencephaly, a prominent NTD, in a subset of embryos. Interestingly, when they examined the exencephalic embryos, they found a pronounced induction of ectopic senescence at early stages, prior to NT closure. This included obvious induction of multiple senescence markers including SA β -gal, p21, p27, gH2AX and H3K9me3 expression, decreased proliferation and SASP activation. Interestingly, similar to the VPA model, senescence here was also detected specifically in the NE cells of the neural tube.

Investigating the molecular regulation of this aberrant senescence further, the authors identified Foxo3a, which was previously linked with diabetes-induced NTDs, as a key inducer of senescence in the NECs [48]. In particular, they showed that Foxo3a regulates the microRNA (miRNA) miR-200c, and identified that miR200c-deficient embryos from diabetic mothers have reduced incidence of senescence and NTDs. So far, the data supported that ectopic induction of senescence genes, regulated by FoxO3a-miR200c axis, was involved in NTD. Critically, they then showed that mice deficient in the senescence mediators p21 or p27 have reduced incidence of senescence and NTDs in diabetic pregnancies. Conversely, they also showed that transgenic expression of p21 and p27 together in the developing NECs was sufficient to induce senescence and the NTD.

Finally, the authors explored the concept that senotherapeutic treatment might diminish the senescence burden and alter the incidence of diabetes-induced NTDs. To address this, they treated diabetic pregnant mice with a regimen of Rapamycin, an FDA-approved drug that can function as a senomorphic in certain contexts [49]. Critically, this resulted in significantly lower levels of senescence, and reduced frequency of NTDs.

Altogether, this important study uncovered a significant role for aberrant senescence in diabetes-induced NTD, and introduced the concept that some developmental birth defects may be amenable to senotherapeutic treatment.

Senescence and Down syndrome

Another recent study also linked premature or ectopic senescence with Down syndrome (DS), a

developmental disorder with a wide range of neurodevelopmental and physical disabilities [50] (Fig. 1C). This is a genetic disorder, most frequently caused by having a full extra, third copy of chromosome 21 (called trisomy 21). In DS patients, some of the neurodevelopmental abnormalities have been linked with alterations in the proper functioning of the NPCs, a derivative of the NECs that will differentiate into neurons, astrocytes and oligodendrocytes [51]. This study investigated nuclear and transcriptional changes in patient-derived iPSCs that were differentiated into NPCs, and how this differed from the original iPSC's. Interestingly, they uncovered that the NPCs from trisomy 21 patients had distinct chromosomal alterations that were not detectable in the original iPSCs. When they profiled the transcriptome of these NPCs, they identified the differentially expressed genes (DEGs) pointed towards an involvement of senescence. Investigating further, indeed they found that cultured trisomy 21 NPCs had increased SA β -gal, increased p16^{Ink4a}, and decreased HMGB1, all indicative of senescence. To further support this connection, they took a bioinformatics approach, and compared the transcriptome of the trisomic NPCs to profiles of senescent cells induced by different approaches. This uncovered a shared signature with oxidative stress-induced senescence, replicative senescence and oncogene-induced senescent cells, which included additional senescence and SASP gene changes. Altogether, this demonstrated that trisomy 21 leads to senescence induction, at least in a sub-population of NPCs.

Next, the authors also asked whether these cells would be sensitive to treatment with senotherapeutics, in this case a combination of compounds, dasatinib and quercetin (DQ), which has senolytic activity in certain settings [26,49]. Here, exposure of the NPCs in culture to a regimen of DQ led to a reduction in senescence markers, including decreased p16^{Ink4a} expression, increased apoptosis, and a significant decrease in global gene expression induced by trisomy 21. Functionally, this also led to increased proliferation, restored to control levels, and improved migratory capacity, which was perturbed by trisomy 21.

In summary, senolytic treatment of NPCs from Down syndrome patients resulted in decreased senescence markers, and improved cell function. Of course, while this study in iPSC-derived NPCs has the caveat that it may not fully model the phenotypes seen during development *in vivo*, it does raise a number of exciting questions and opportunities regarding the potential contribution of aberrant senescence to this spectrum of developmental defects.

Future directions

Other models to investigate a potential senescence-involvement

As these studies have now uncovered the involvement of aberrantly induced cellular senescence in the pathogenesis of developmental birth defects, this raises the exciting possibility that it might be involved in other developmental syndromes and defects. But is it possible to predict which diseases might be affected? Perhaps not surprisingly, reviewing the broader literature makes it quite easy to identify many developmental defects where senescence might be examined, but where perhaps it was not considered.

Curiously, the studies above all resulted in various neurodevelopmental defects including NTDs, microcephaly and exencephaly. As NTDs are among the most common congenital birth defects, this raises the concept that senescence may play a previously unrecognized role in the pathogenesis of these defects. Indeed, there are over 200 gene mutations that can cause NTDs, often through misregulation of NEC fate and function, including impaired neural fold elevation and altered cell death [52]. The inclusion of an assessment for possible altered senescence in these models could uncover additional and important mechanistic insight.

Another interesting observation from these studies is a preponderance to senescence activation in the NECs. Does this suggest that these cells are particularly sensitive to senescence-inducing signals? In both the VPA and diabetes models discussed above, senescence caused a block in neural differentiation, with similar effects on the iPSC-derived NPCs from trisomy 21 patients (Fig. 2). In humans, such effects of blocking or even disrupting neurogenesis at different stages could manifest in a wide variety of effects, depending on the severity and timing of induction. With regards to VPA-exposure *in utero*, the predominant effect is neurodevelopmental delay, including cognitive defects and ASD-like features. The association here of a robust senescence response supports that further studies should be undertaken to explore potential links with embryonic or early-postnatal senescence and ASD in additional models.

Also, although VPA-exposure induced microcephaly, a condition where the circumference of the head is smaller than normal at birth, microcephaly is most frequently caused by inherited genetic mutations, by exposure to environmental toxins, or viral infection [53]. Indeed, mutations in a number of genes involved in the DNA-damage response (DDR) and centrosome

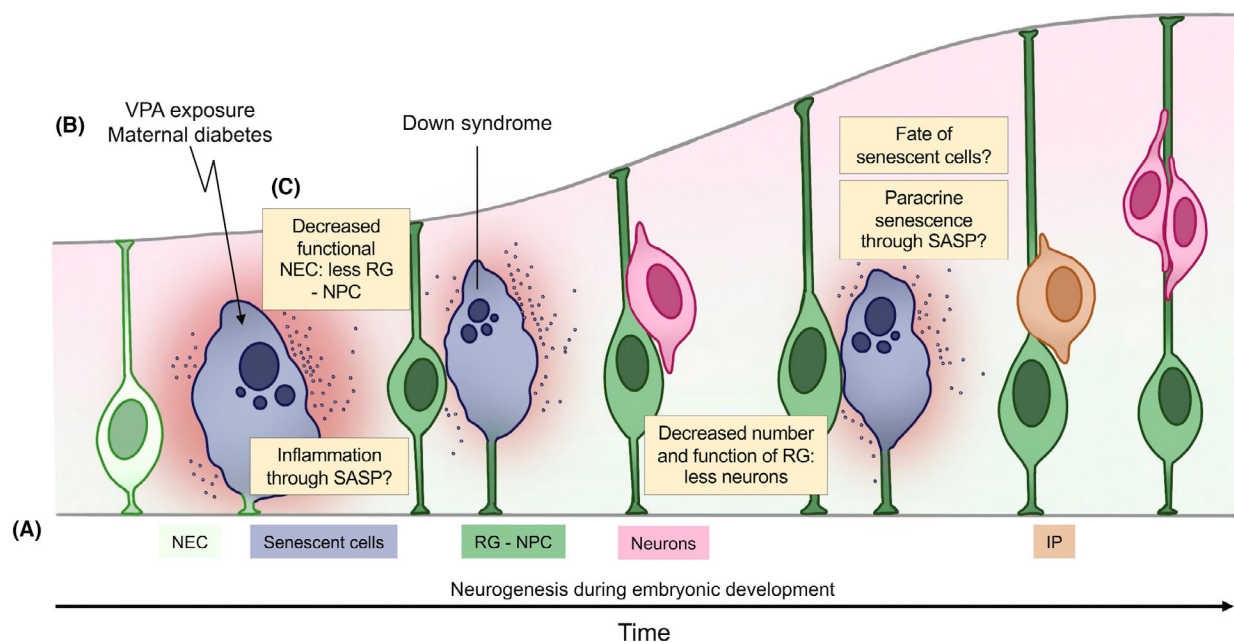


Fig. 2. Proposed mechanisms by which ectopic senescence impacts early neurodevelopment. (A) Scale from left to right represents sequential differentiation occurring during embryonic neurogenesis. The initial cells of the nervous system are NECs forming the neural tube. At onset of neurogenesis, NECs transition to RG cells. Radial glial cells are early NPCs (also called apical progenitors), and can divide symmetrically to form more RG, or asymmetrically to generate neurons. In some regions, RG give rise to intermediate BPs, which then form neurons. Scale from top to bottom represents the thickness of the cortex. (B) The studies discussed in the text reported ectopic senescence in NEC [35,46] and in NPC [50]. (C) Hypothetical impacts of senescence during neurogenesis. NEC and NPC becoming senescent no longer proliferate and fail to generate subsequent cell types. SASP secretion could also increase inflammation, possibly impairing the functionality of the tissue or triggering paracrine senescence in all cell types of the nervous system. Each of these outcomes could lead to a decreased number of mature neurons or other cell types. Moreover, senescent cells and their SASP could also disrupt additional embryonic pathways or the integrity of the tissue.

mutations have been linked to microcephaly [53,54]. Whether mistimed or ectopic senescence might be implicated remains to be investigated. In addition, as seen with the recent outbreak, Zika virus infection also causes pronounced microcephaly, which involves induction of cell death and decreased growth in cerebral organoids [55,56]. As viral infection can be a trigger for senescence [57], it will also be interesting to explore whether Zika virus-induced microcephaly may also involve ectopic senescence.

Seckel syndrome is a rare human disease characterized by microcephaly and dwarfism [54,58]. Mouse models of ATR-mutations, one cause of this disease, also exhibit premature ageing. Interestingly, one study traced the cause back to the embryo, where increased replicative stress led to pronounced DNA-damage and p53-expression [58]. As replicative stress is one inducer of senescence, it might be interesting to assess senescence in this model.

DNA damage and genotoxic stress is also a feature of Cornelia de Lange syndrome (CdLS), a

developmental disorder that affects many tissues, including limb, gut, heart and brain development. This is caused by mutations in cohesin, a protein complex that is required for chromosome segregation, genome maintenance and DNA damage repair. Interestingly, a recent study made an exciting connection with the embryonic disorder and aberrantly induced senescence resulting from persistent DNA damage and the disease, but this time in the placenta [59]. While senescence has been described in the placenta [60], CdLS mutations exacerbated this, resulting in increased cytokine signalling that perturbed embryonic development. Remarkably, replacement of the mutant placenta with a wild-type one, or inhibition of cytokine signalling, partially rescued the defects, demonstrating how mis-regulated senescence outside of the embryo can also contribute to developmental disorders.

Somatic mutations in the Ras gene are well-characterized inducers of oncogene-induced senescence. However, germline mutations in Ras and other genes that control or mediate the Ras/Map kinase (Ras/

MAPK) pathway are the cause of a major class of developmental defects known as RASopathies [61]. These include neurofibromatosis, Noonan and Costello syndromes among others, and affect multiple tissues depending on the specific mutation, but alterations in the craniofacial tissue, heart, skin, bones, eyes, and overall size are common. Given the association with hyperactivation of Ras signalling, possible links with aberrant induction of embryonic senescence is worth investigating.

The tumour suppressor gene p53 is a master regulator of senescence and the SASP, and aberrant induction of p53 in the embryo has been implicated in a wide variety of developmental syndromes, some of which have been elegantly modelled in mice [62,63]. These include defects in multiple developing tissues including neuronal defects, exencephaly, heart and eye defects among others. As decreased proliferation is one of the associated embryonic features, it would also be interesting to assess a potential senescence involvement. In a similar manner, point mutations in the p53-family member p63 causes Ectrodactyly Ectodermal Dysplasia Cleft Lip/Palate (EEC) syndrome [64]. As p63-deficiency induces senescence [65], it will also be worthwhile to investigate whether senescence might in some part contribute to some of the EEC-associated phenotypes.

Inflammation has been linked with a wide variety of neuro/developmental and early-life disorders, including ASD, cognitive impairment, cerebral palsy, and others. Senescent cells can act as potent contributors to tissue inflammation, or vice versa, inflammatory factors can induce senescence arrest. In models of each of these disorders, it will be worthwhile to check if there may be an aberrant induction of senescence during development.

Of course, these are only some suggestions of the many models or disease states where the induction of senescence might be investigated. No doubt there are others, including numerous rare diseases.

Conclusion and remarks

Outstanding questions on senescence and developmental defects

The studies highlighted above introduce the concept of how ectopic senescence can contribute to a number of developmental defects. Previous studies have described how programmed, timed senescence is present in normal embryos in specific tissues and at different stages, including the apical ectodermal ridge, the hindbrain roofplate, the mesonephros and the endolymphatic

sac. This raises the possibility that senescence contributing to birth defects may potentially arise either through mis-timed or altered activation of the endogenous senescence program, or through aberrant induction of ectopic senescence. Interestingly, in the three studies discussed above, it appears that ectopic damage-induced senescence is activated in cells that would normally not express senescent features, while in the CdLS model, it appears that the mutations increase the levels of endogenous senescence.

As discussed above, these studies raise the intriguing possibility that additional developmental defects may also involve an aberrant induction of senescence (either mis-timed or ectopic), perhaps even with a causative role, that has simply gone unnoticed because it was never assessed. While the analysis of proliferation and cell death is quite common in various models of developmental disorders, the analysis of senescence has usually not been considered. This is likely because of the primary association of cellular senescence with ageing and age-related diseases. However, based on these emerging findings, it now appears that it is also worth to assess for mis-timed senescence induction. As shown in these studies, this could include assessing for SA β -gal activity, either at the whole-mount level for early-stage embryos, or on tissue sections in later stages. In addition, patient-derived organoids represent a powerful emerging tool to explore this link. Expression of senescence genes can also be measured, checking for elevated or mis-timed expression of genes such as p21, p16^{Ink4a}, p19^{Arf}, or SASP genes. Ideally then functional intervention, either with genetic loss-of-function approaches, or potentially even senotherapeutic strategies could prove highly informative.

Of course, the level of senescence induction may play additional complicating roles. In the models described above, there are varying levels of senescence induced in different embryos that may correspond with variations in phenotype; perhaps a smaller burden of senescence in an embryo induces milder effects, while a high burden associates with more severe phenotypes. However, small, and perhaps transient induction of senescence could be quite difficult to detect. Therefore, the study of senescence in developmental defects will benefit significantly from the use of new tools such as senescence reporter vectors or mouse models which can provide a quantitative readout of senescence burden.

Another outstanding question relates to the fate of these senescent cells. In many situations, including during normal embryonic development, in premalignant lesions, and in tissue damage, senescent cells are actively removed by the immune system, while it is

thought that impaired removal of senescent cells leads to their accumulation during ageing [7,18,28]. In the models of developmental defects, it was not demonstrated whether the senescent cells are removed by the immune system or persist through later stages of development and in postnatal tissue, thus the ultimate fate of the senescent cells remains unclear (Fig. 2). In the diabetic embryopathy model, senescence was associated with increased macrophage infiltration in some embryos [46], suggesting that it is likely they are also cleared by immune cells, but additional studies are needed to verify how effective this might be. If senescent cells are not efficiently removed during development, it is conceivable that the persistence of these highly inflammatory cells could have significant and continued effects on the developing or even postnatal tissues. Again, the generation and application of new senescence tools such as reporter- and tracing-models will prove invaluable.

Recent studies are uncovering additional evidence regarding the heterogeneity of senescence in adult tissues and disease models. While it was once considered that senescent cells may follow a dynamic program with sequential activation of senescence-mediating genes, new evidence suggests the situation is more complex, for example with p21 and p16^{Ink4a} being expressed in different cells types in certain contexts such as in ageing and tissue damage [66,67]. Such information should also be taken into consideration when investigating senescence during embryogenesis and in developmental defects. For example, during normal development, it is suggested that p21 is a primary mediator, with little or no expression of p16^{Ink4a} or p19^{Arf}. While in the disease-models studied, VPA mediates NE senescence via p19^{Arf}, while diabetic embryopathy involves p21/p27, and DS appears to involve p16^{Ink4a} expression. This further highlights how senescence can be induced in different ways, by different signals. Future studies incorporating single-cell sequencing, or spatial transcriptomics will prove highly informative in identifying which senescence genes and SASP factors are induced in different cell types and disease models.

Such implications of senescence as playing a causative role in developmental defects naturally raise the question of whether these might be targeted with senotherapeutics, either through killing senescent cells (senolytics) or altering their properties (senomorphics). Indeed, the model of diabetic embryopathy provides exciting support for this, demonstrating reduced incidence of senescence and NTDs in response to Rapamycin treatment [46]. Additionally, as treatment of trisomy 21 NPCs in culture with the senolytic DQ also

resulted in beneficial effects, it will be very exciting to see how treatment affects *in vivo* models, or organoids with trisomy 21 [50]. These results indeed suggest that other developmental defects where senescence is identified may also be targeted. For example, although not examined in the study discussed above, VPA-induced defects may also be amenable to senotherapeutic manipulation. A recent study demonstrated that VPA-induced gene-expression signatures are reversed by treatment with Cardiac Glycosides (CGs) [68]. Interestingly, recent reports have identified that various CGs can act as potent senolytics, raising the exciting possibility that these might be tested *in vivo* to perturb VPA-induced developmental senescence [69,70]. Given the ever-increasing list of compounds that alter senescence in adult and aged states, it is exciting to think that some of these may also work *in utero*. Of course, any senotherapeutic approach will have to take into consideration whether targeted manipulation of senescence in disease situations might inadvertently perturb the endogenous senescence program in the embryo. While this did not appear to be a problem with DQ treatment in the diabetic embryopathy model, additional care and assessment will be needed to evaluate and rule out any potential side-effects.

Cellular senescence is a dynamic cellular state induced by a variety of stimuli, including cell and tissue damage, development and regeneration cues, and ageing. Here, we emphasize how this is a cellular process, that when aberrantly activated, can have significant repercussions on the developing embryo. As senescence is often used in reference to the general process of organismal ageing, this has probably not encouraged developmental biologists to examine for this cellular process in their models. Hopefully now, with increased awareness, additional disease models affected by cellular senescence can be identified.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

All authors contributed to the writing, editing and review of the manuscript.

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