Two starting questions:

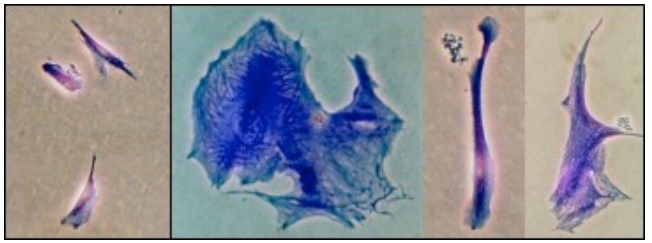
- What is cellular senescence?

- What are the cell / tissue level hallmarks of ageing?

Cell Senescence

Growth arrest

Increase in size



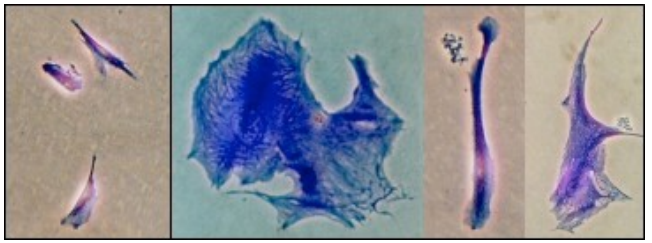
Young old old old

Senescence-associated β-galactosidase (SA β-gal) activity

Cell Senescence

Growth arrest

Increase in size



Young old old old

Senescence-associated β-galactosidase (SA β-gal) activity

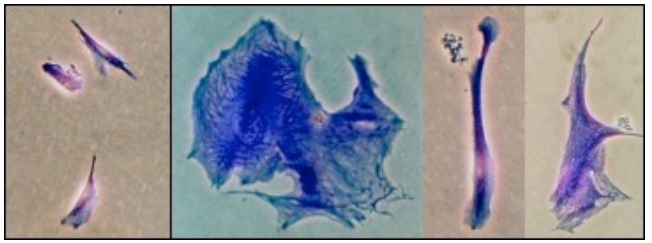
Pro-inflammatory cytokine release

INFLAMM-AGEING

Cell Senescence

Growth arrest

Increase in size



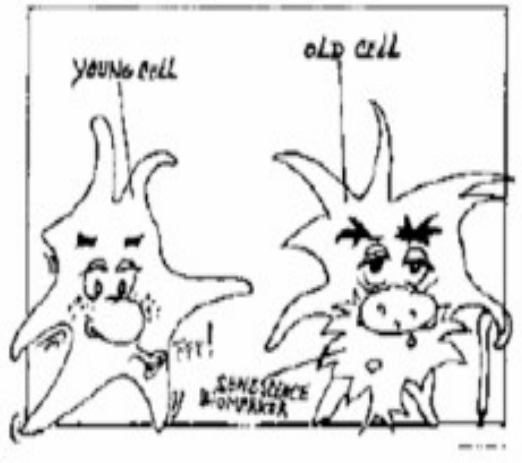
Young old old old

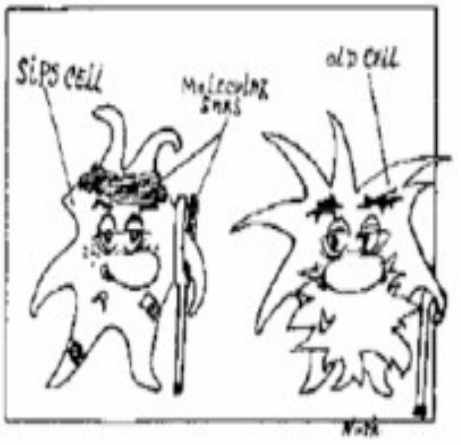
Senescence-associated β-galactosidase (SA β-gal) activity

Pro-inflammatory cytokine release

INFLAMM-AGEING

Stress-induced premature senescence:

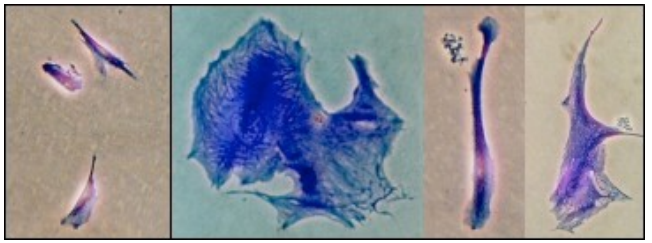




Cell Senescence

Growth arrest

Increase in size



Young old old old

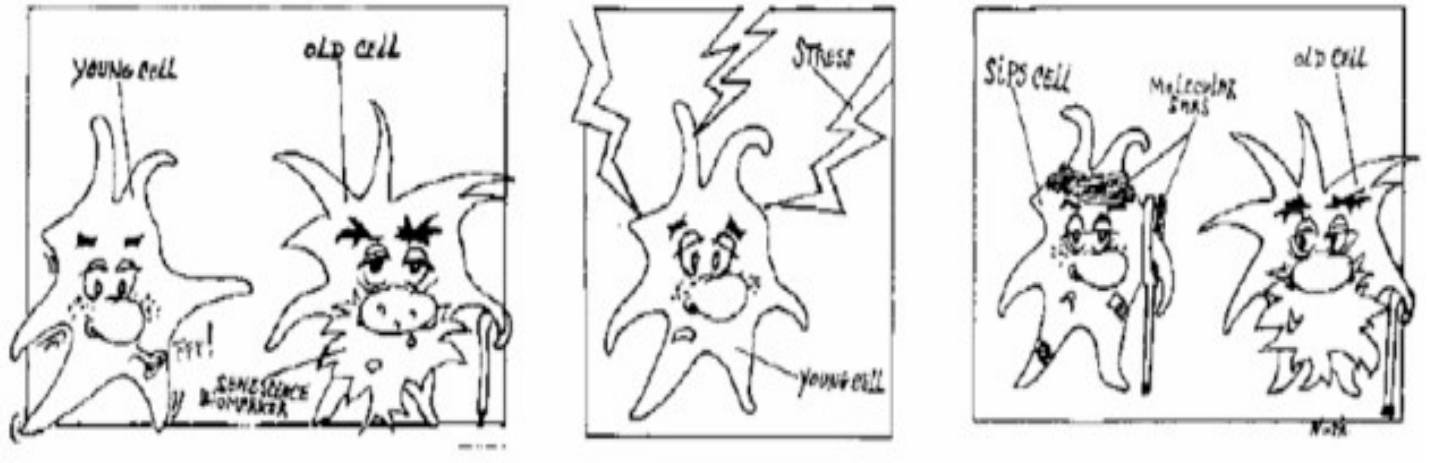
Senescence-associated β-galactosidase (SA β-gal) activity

Pro-inflammatory cytokine release

INFLAMM-AGEING

Needs p21

Stress-induced premature senescence:



Both in Cell, Nov 2013

Cell

1

Programmed Cell Senescence during Mammalian Embryonic Development

Daniel Muñoz-Espín,[1] Marta Cañamero,[2] Antonio Maraver,[1] Gonzalo Gómez-López,[3] Julio Contreras,[4,5,6] Silvia Murillo-Cuesta,[5,6] Alfonso Rodríguez-Baeza,[7] Isabel Varela-Nieto,[5,6] Jesús Ruberte,[8] Manuel Collado,[1,9] and Manuel Serrano[1,*]

Cell

2

Senescence Is a Developmental Mechanism that Contributes to Embryonic Growth and Patterning

Mekayla Storer,[1] Alba Mas,[1] Alexandre Robert-Moreno,[1] Matteo Pecoraro,[1] M. Carmen Ortells,[1] Valeria Di Giacomo,[1] Reut Yosef,[2] Noam Pilpel,[2] Valery Krizhanovsky,[2] James Sharpe,[1] and William M. Keyes[1,*]
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http://dx.doi.org/10.1016/j.cell.2013.10.041

Both of these groups looked for any signs of senescence in embryos.

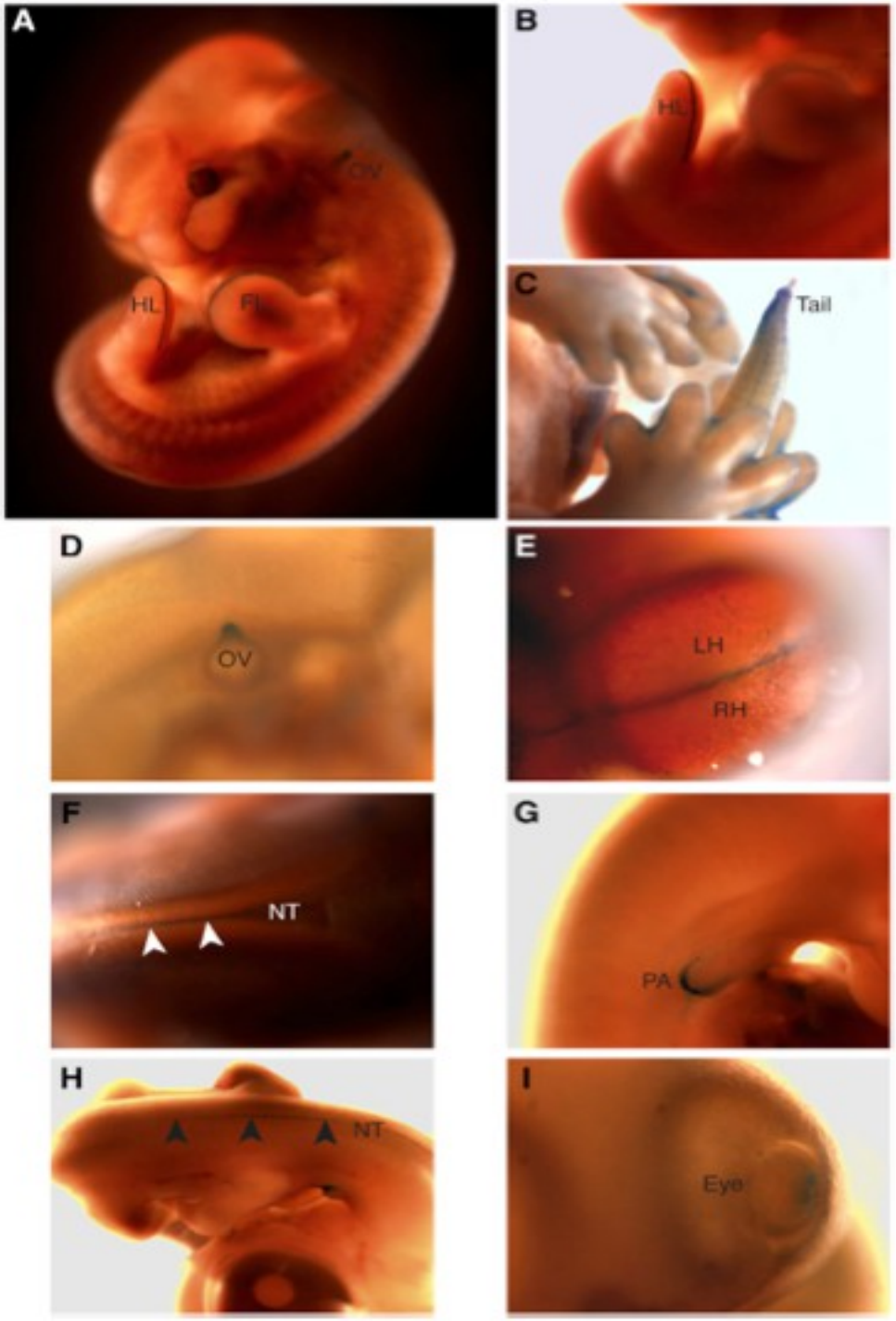


Figure 1. Senescence-Associated β-Galactosidase Is Expressed in Many Tissues during Embryonic Development

Mouse and chick embryos were stained at the whole-embryo level for SAβ-gal. Representative images of regions that stained positive are shown.

(A) Mouse embryo, E11.5.

(B) mouse hindlimb, E11.5.

(C) The distal tip of the mouse tail, E14.5.

(D) Mouse otic vesicle, E10.5.

(E) Fusion zone of the vesicles of the mouse hindbrain, E11.5.

(F) Fusing neural tube of the mouse, E11.5.

(G) Chick pharyngeal arches, Hamburger and Hamilton (HH) stage 28.

(H) Chick neural tube, HH28. Arrows denote the line of positive staining along the length of the neural tube.

(I) Chick eye, HH28.

Total numbers examined at each stage listed in Table S1. OV: otic vesicle, HL: hindlimb, FL: forelimb, LH: left hemisphere, RH: right hemisphere, NT: neural tube, PA: pharyngeal arch. See also Figure S1 and Table S1.

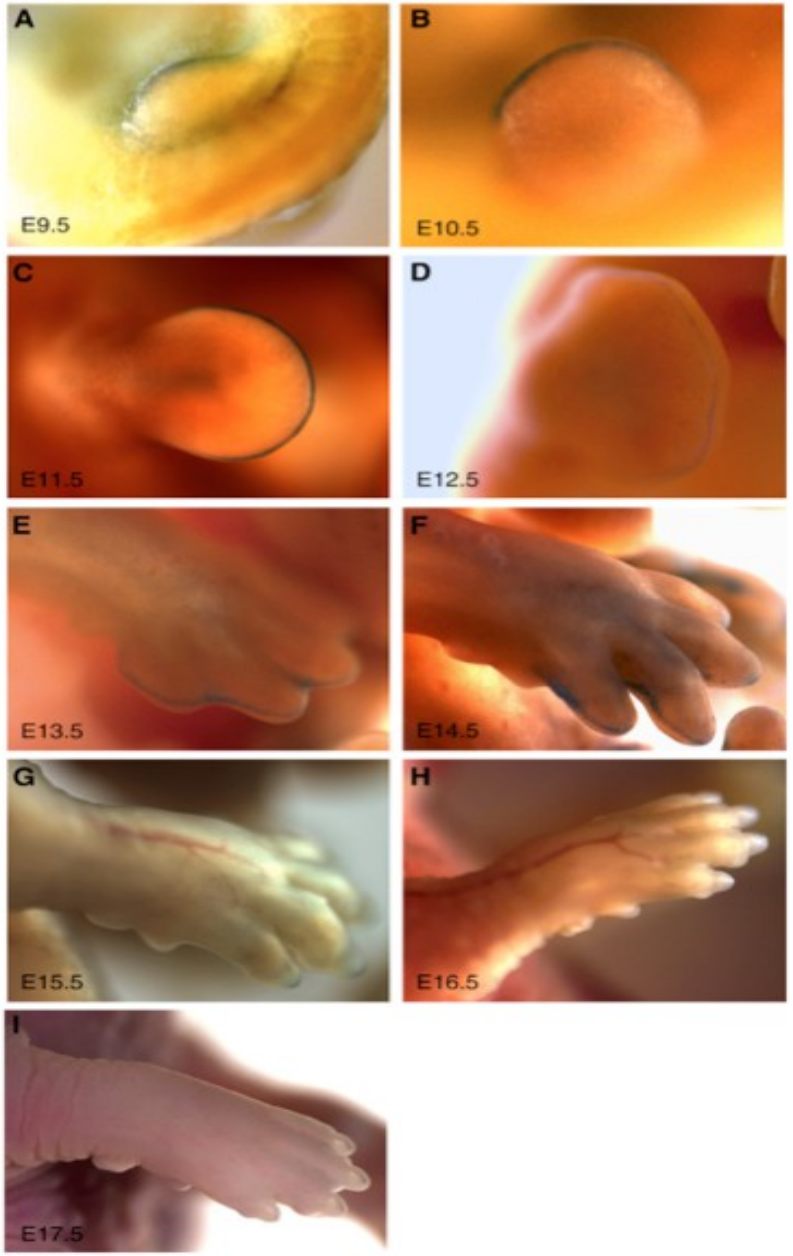


Figure 5. Timeline of Senescence during Mouse Forelimb Development

SAβ-gal staining showing the localization of senescent cells during mouse forelimb development. (A) E9.5; (B) E10.5; (C) E11.5, showing prominent staining in the AER; (D) E12.5; (E) E13.5; (F) E14.5; (G) E15.5; (H) E16.5; (I) E17.5. See also Figure S5.

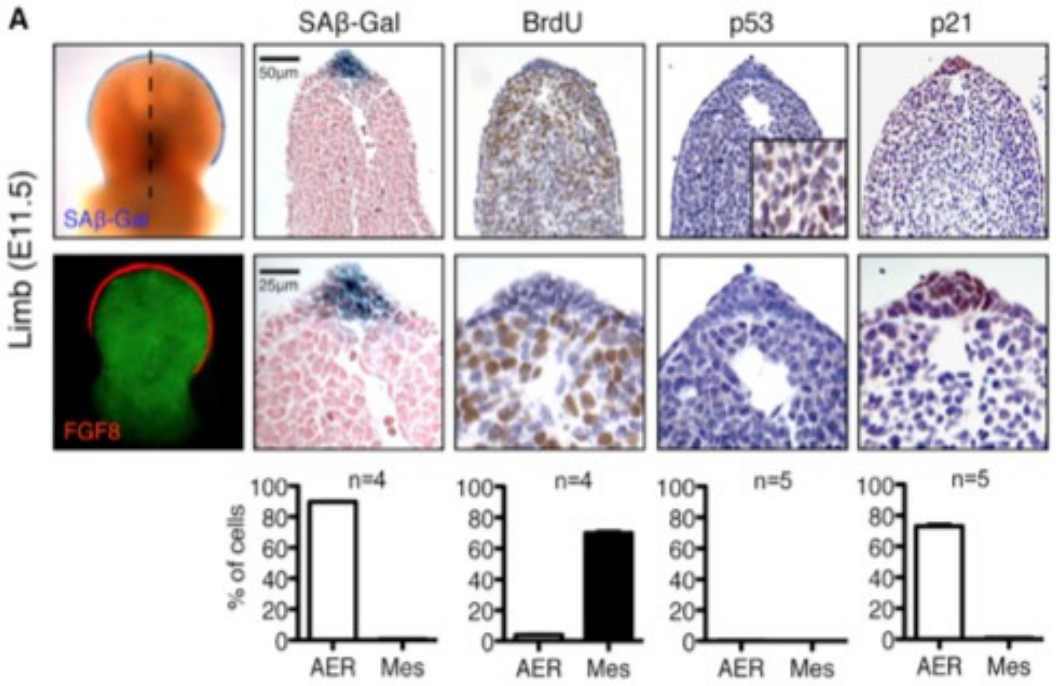


Figure 2. Senescence Markers Are Expressed in the Developing Forelimb and Neural Tube

(A) Forelimb stained for SAβ-gal, BrdU incorporation, p53, and p21 by immunohistochemistry. The insert panel of positive p53 staining corresponds to limb mesenchymal tissue of irradiated wild-type (WT) embryos. Top left image shows SAβ-gal whole-mount staining with a line showing the plane of section for the staining. Bottom left image was processed for in situ hybridization with FGF8 and scanned by OPT to allow visualization of the AER. The graphs at the bottom show the percentage of positively stained cells in the AER and underlying mesenchyme (mes).

What do these sites have in common?

Reminder – they included things like..

Apical ecodermal ridge of limb bud
Tip of tail
Otic vesicle
CNS centre-line

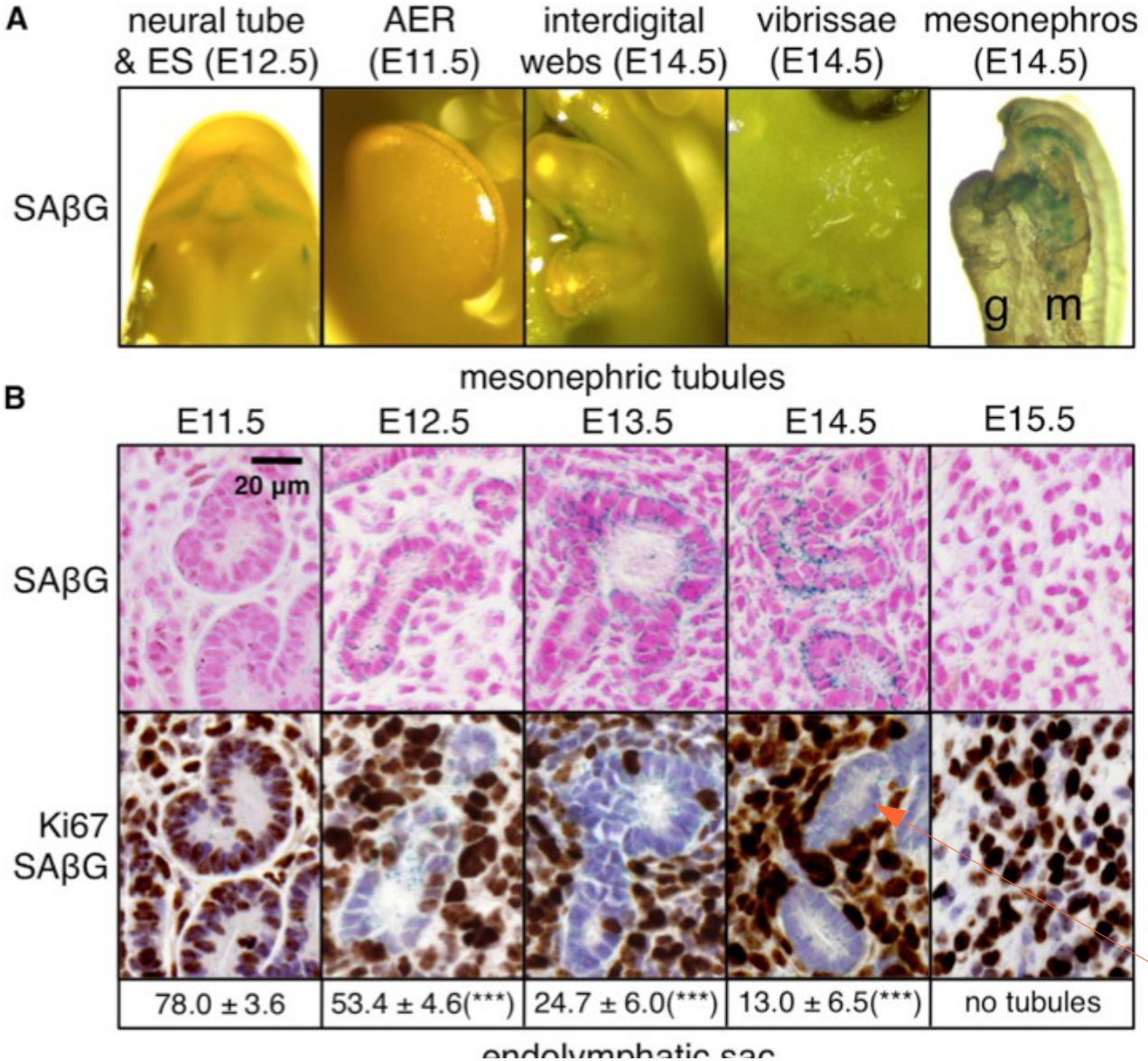


Figure 1. Programmed Senescence Occurs during Mouse Embryonic Development

(A) Examples of senescent structures after embryo whole-mount SAβG staining at the indicated stages. ES, endolymphatic sac; AER, apical ectodermal ridge.

(B) Whole-mount SAβG staining and Ki67 immunohistochemistry of mesonephric tubules at the indicated stages. The percentages of Ki67-positive cells in the mesonephric tubules are shown (E11.5, n = 4; E12.5, n = 3; E13.5, n = 3; E14.5, n = 7).

Ki67 marks proliferation

(Note how senescence and proliferation do not overlap).

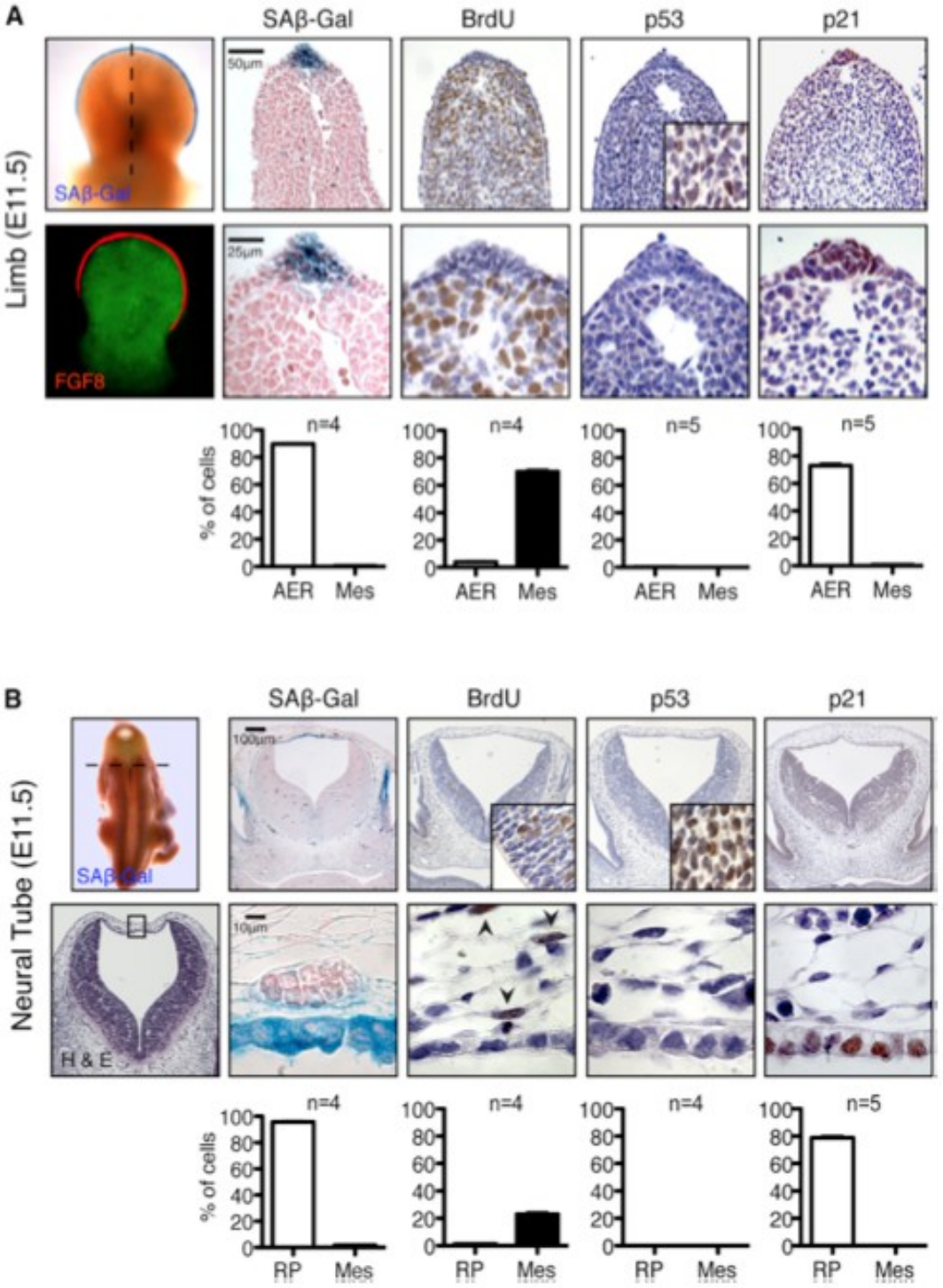
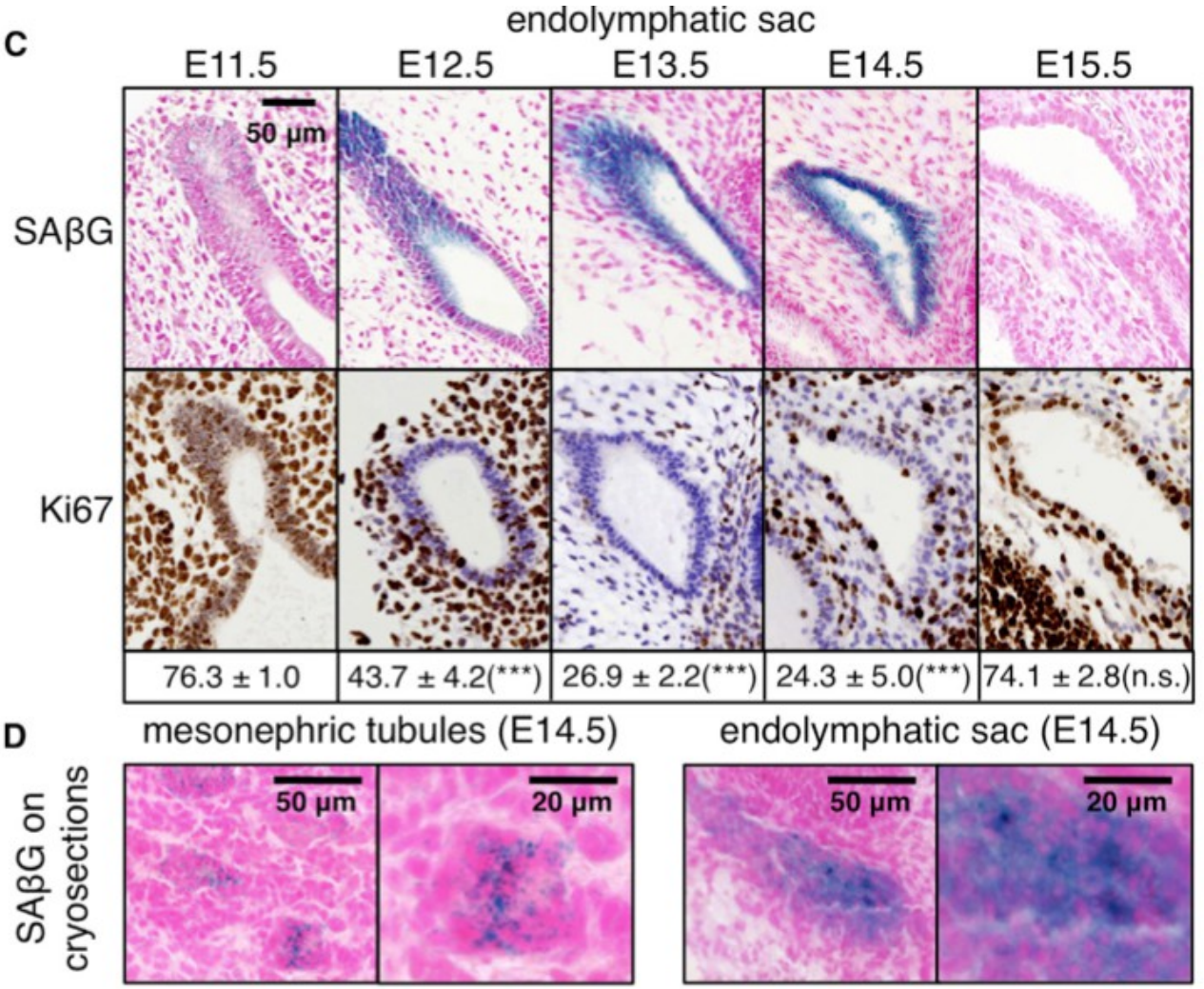


Figure 2. Senescence Markers Are Expressed in the Developing Forelimb and Neural Tube

(A) Forelimb stained for SAβ-gal, BrdU incorporation, p53, and p21 by immunohistochemistry. The insert panel of positive p53 staining corresponds to limb mesenchymal tissue of irradiated wild-type (WT) embryos. Top left image shows SAβ-gal whole-mount staining with a line showing the plane of section for the staining. Bottom left image was processed for in situ hybridization with FGF8 and scanned by OPT to allow visualization of the AER. The graphs at the bottom show the percentage of positively stained cells in the AER and underlying mesenchyme (mes).

(B) Left panels show whole-mount embryo highlighting plane of section, and below, box indicates area shown in high-magnification images. Neural tube stained for SAβ-gal, BrdU incorporation, p53, and p21 by immunohistochemistry. The BrdU insert corresponds to an enlarged area in the neural tube. The graphs at the bottom show the percentage of positively stained cells in the neural RP and adjacent mesenchyme (mes).

All samples stained were WT embryos at E11.5. Values are expressed as mean ± SEM; n = total number of embryos analyzed. See also Figure S2.



(C) Whole-mount SAβG staining and Ki67 immunohistochemistry of the endolymphatic sac at the indicated stages. The percentages of Ki67-positive cells at the endolymphatic sac are shown (E11.5, n = 3; E12.5, n = 5; E13.5, n = 5; E14.5, n = 10; E15.5, n = 4). (D) Cryosections containing mesonephric tubules (left) and endolymphatic sac (right) stained with SAβG.

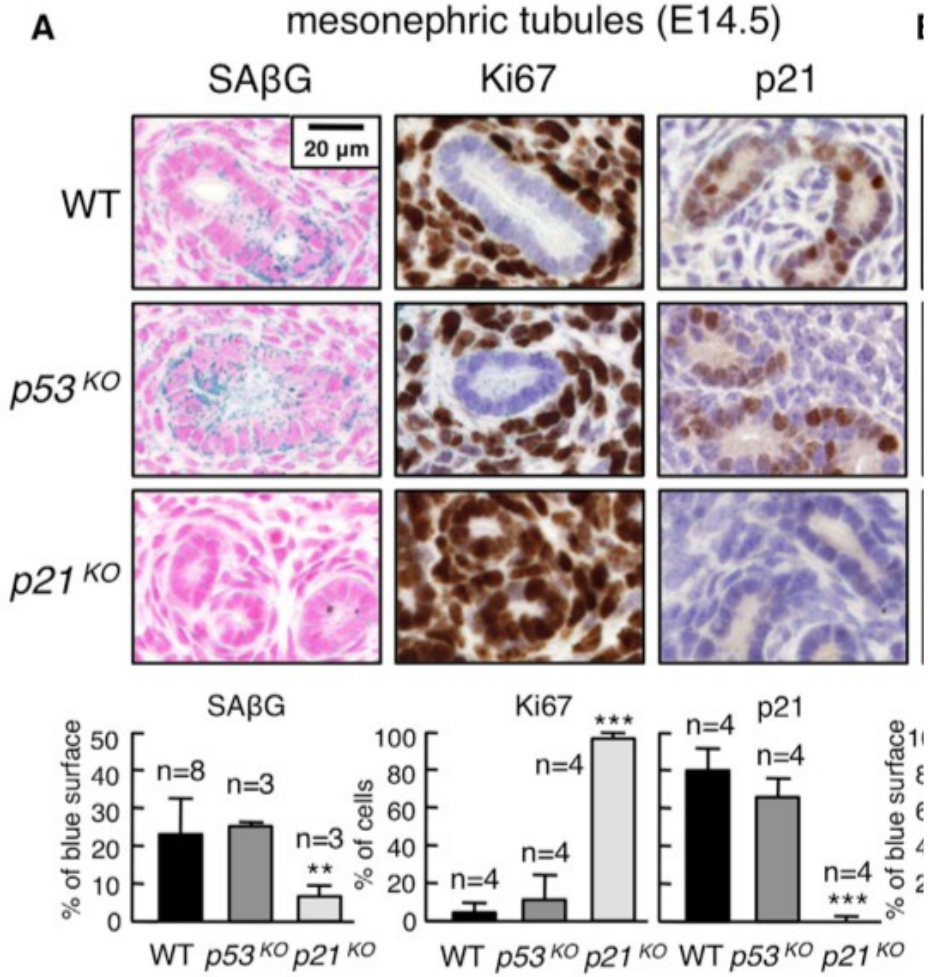


Figure 2. Expression of Cell-Cycle Inhibitors in the Mesonephric Tubules and Endolymphatic Sac

(A) Mesonephric tubules stained for p53, p21, p27, or p15. The graphs show the percentages of positively stained cells in the epithelia of the mesonephric tubules at E11.5 and E14.5. Dotted black lines, at E11.5, separate the mesonephros (upper part) from the gonad (lower part).

(B) Endolymphatic sacs stained as in (A). The graphs show the percentages of positively stained cells in the endolymphatic sac epithelia at E11.5 and E14.5. In the case of p15 staining, the graph shows the percentage of positively stained cells at both epithelial (sac) and perisacular (perisac) regions at E11.5 and E14.5. The picture exhibiting p15 staining at E14.5 corresponds to an embryo subjected to whole-mount SAβG staining to differentiate the epithelium from the perisacular region.

All samples correspond to WT embryos. Values are expressed as mean ± SD, and statistical significance was assessed by the two-tailed Student's t test: *$p < 0.05$; ***$p < 0.001$; n.s. (not significant); the total number of embryos analyzed is indicated in each graph (n).

See also Figure S2.

Any thoughts about what might be going on here?

… about the nature of the relationship between cell senescence and development?

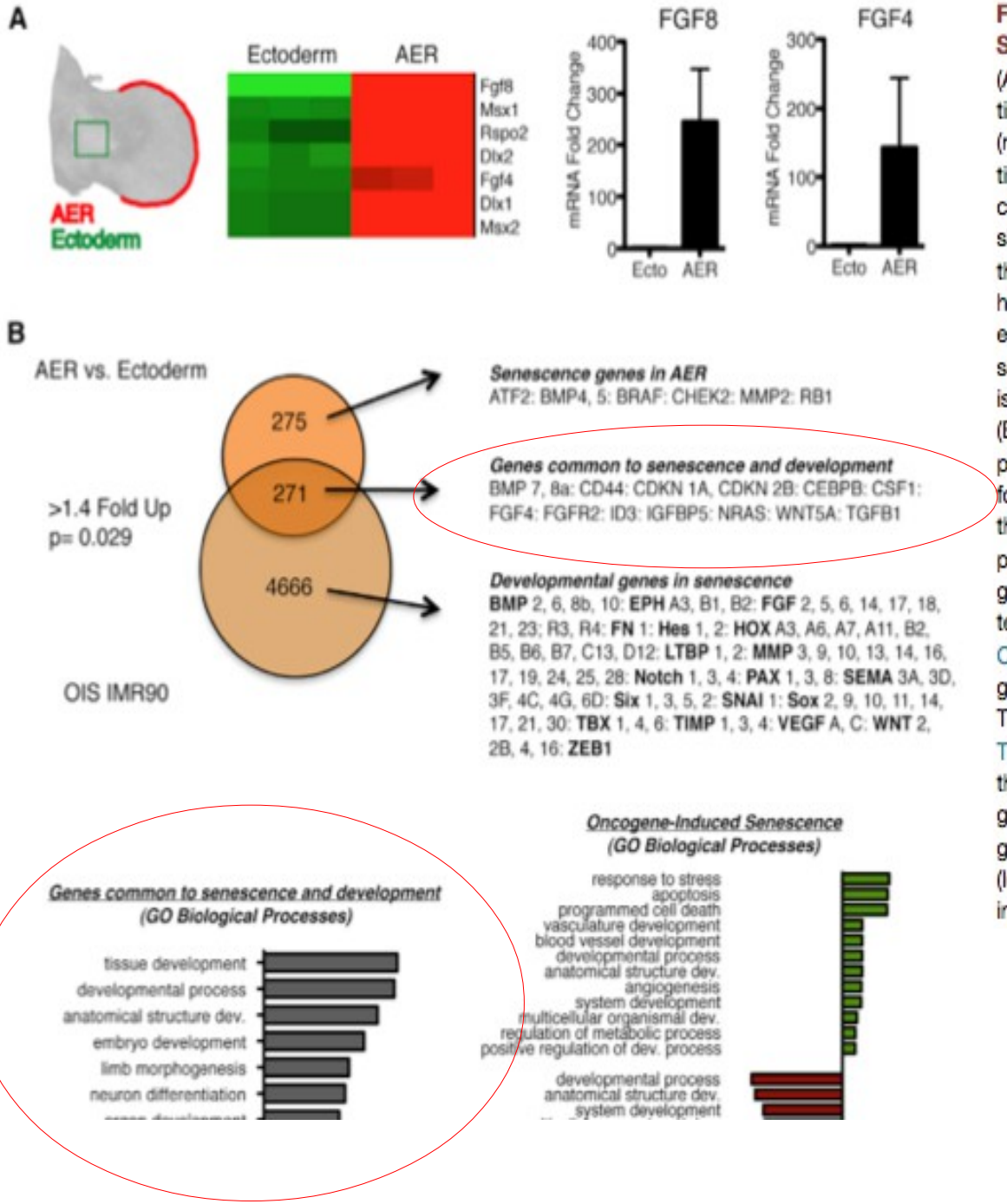


Figure 3. Developmental Senescence Shares a Molecular Signature with OIS

(A) Schematic demonstrating regions in which tissue samples were collected for microarray (red = AER, and green = ectoderm). Representative heatmap of the microarray profile on AER compared to nonsenescent adjacent ectoderm is shown. Example genes are shown to demonstrate that known markers of the AER were expressed at higher levels (red) in the AER compared to the ectoderm (green) (left). qPCR expression on separate biological replicates for FGF8 and FGF4 is shown. Values are expressed as mean ± SEM.

(B) Representational overlap of the microarray profile of gene probes that were upregulated >1.4-fold in the AER versus ectoderm compared to those that were upregulated >1.4-fold in two pooled arrays of IMR90 human fibroblasts undergoing OIS. Statistical analysis denotes Fisher's test of enrichment, p = 0.029. OIS data are from Collado et al., 2005. Representative examples of genes from each section are denoted with arrows. The full list of genes and their overlap is shown in Table S2, along with the AER expression values for the genes common to OIS and AER. Underlying graphs represent GO analysis of upregulated genes common to senescence and development (left), downregulated genes (Figure S3), and genes in OIS (right).

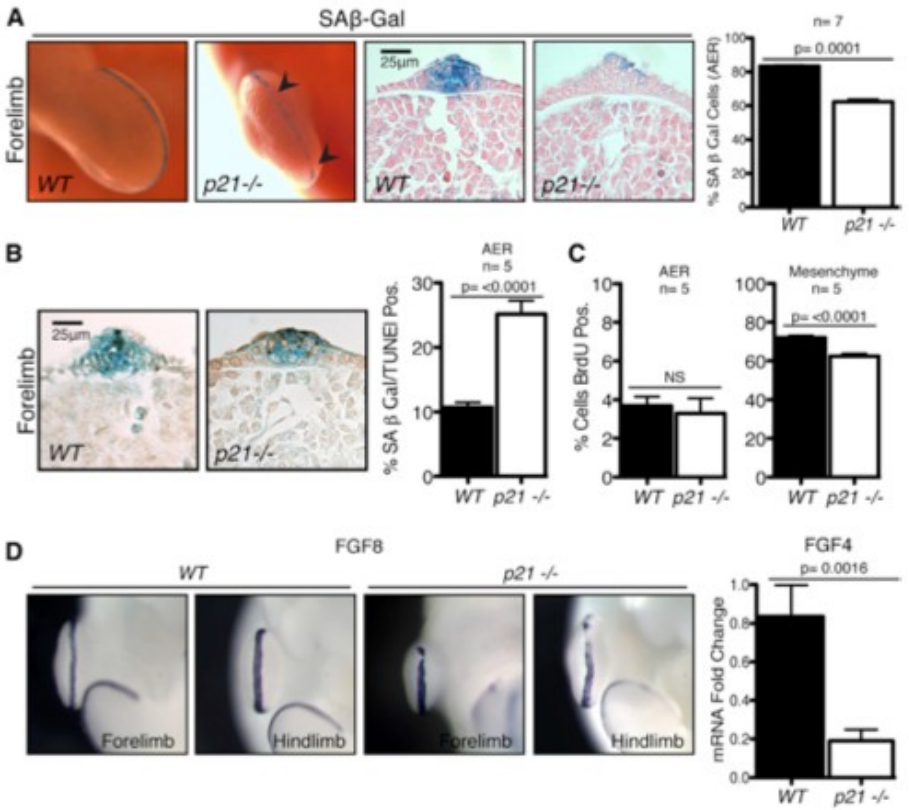


Figure 4. p21 Deficiency Impairs Developmental Senescence and Induces Patterning Defects in Limbs and OIS Cells

(A) Representative images of limbs from WT and p21-deficient embryos that had been stained at the whole-embryo level for SAβ-gal at E11.5 and corresponding sections. Graph shows percentage of SAβ-gal-positive cells in the AER in WT and p21-deficient embryos (n = 7 embryos; values are ± SEM, t test).

(B) Sections of whole-mount stained SAβ-gal embryos, costained with TUNEL. Graph shows percentage of the SAβ-gal population that are TUNEL positive in the AER in WT and p21-deficient embryos (n = 5 embryos; values are ± SEM, t test).

(C) Graphs shows the percentage of BrdU incorporation in the AER and mesenchyme in WT and p21-deficient embryos (n = 5 embryos; values are ± SEM, t test).

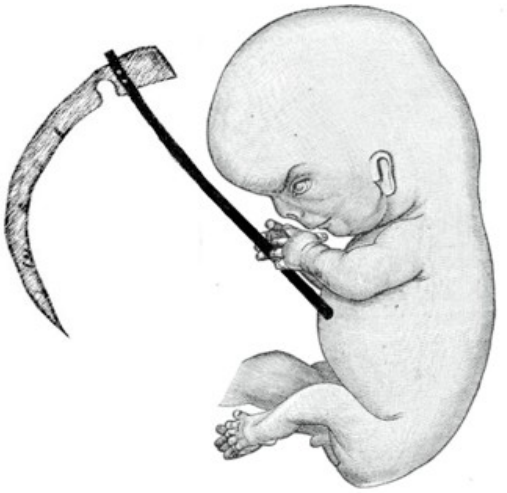
(D) In situ hybridization for FGF8 on forelimbs and hindlimbs of WT and p21-deficient mice at E11.5. qPCR for FGF4 expression in the AER from WT and p21-deficient embryos is shown (n = 5 embryos; values are ± SEM, t test).

So can 'senescence' be just a coincidence?

An intriguing possibility:

Our short-lived ancestors evolved a 'signalling centre' cell state: no division, secretion of factors such as TGFb, BMPs, CD44 etc, for developmental purposes

When we became long-lived, we needed to find a way of stopping damaged cells dividing.



Damage detection got connected to the 'signalling centre' state

→ damage stops cell division :-)

→ damage causes cytokine release,
and inflamm-ageing :-(

T. S. Eliot

"East Coker," from *The Four Quartets*

I.

In my beginning is my end. In succession
Houses rise and fall, crumble, are extended,
Are removed, destroyed, restored, or in their place
Is an open field, or a factory, or a by-pass.
Old stone to new building, old timber to new fires,
Old fires to ashes, and ashes to the earth
Which is already flesh, fur, and faeces,
Bone of man and beast, cornstalk and leaf.
Houses live and die: there is a time for building
And a time for living and for generation
And a time for the wind to break the loosened pane
And to shake the wainscot where the field mouse trots
And to shake the tattered arras woven with a silent motto.

  In my beginning is my end. Now the light falls
Across the open field, leaving the deep lane
Shuttered with branches, dark in the afternoon,
Where you lean against a bank while a van passes,
And the deep lane insists on the direction
Into the village, in the electric heat
Hypnotized. In a warm haze the sultry light
Is absorbed, not reflected, by grey stone.

...