Chapter 7

Markers of cellular senescence and chronological age in various human tissues: a systemic review of the literature

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In preparation

Abstract

Background: Cellular senescence, a stable growth arrest of cells, is increasingly recognized as a driver of the aging process. Several studies report higher numbers of senescent cells in a variety of tissues of older humans when compared to the young.
Objective: To systemically describe the literature on the association between markers of cellular senescence and chronological age in different types of tissues.
Methods: We searched Pubmed, Web of Science and Embase for articles that reported on senescence markers dependent on age in any human tissue. Out of 3833 unique articles 43 articles reporting on this topic were identified, including 44 cohorts. Data was extracted on the origin of tissue, the type of markers being used, and the age and gender distribution of the donors. A total of 78 associations between senescence markers and age were reported.
Outcomes: Cohort sizes ranged from 3 to 176 donors, and varied widely in their age distribution. Out of the 78 associations, 34 were positive and statistically significant associations ($p<0.05$) between senescence markers and chronological age, six showed positive trends ($0.05<p<0.10$), 27 associations were inconclusive ($p>0.10$) and one association showed a negative statistically significant association ($p<0.05$). A large proportion of the positive associations were based on studies conducted in blood, whereas it was less often the case in kidney and skin.
Conclusion: Almost half of the associations between markers of cellular senescence and age show a positive significant association. This can be interpreted as proof of an evident biological phenomenon but it is unclear to what extent publication bias explains for these outcomes.

Introduction

Cellular senescence, a stable growth arrest of the cell, has been widely studied *in vitro*. This growth arrest of senescent cells functions as an effective anti-tumor mechanism at the cost of a diminished capacity to regeneration of tissues [1]. Furthermore, senescent cells have been shown to secrete proteins that disrupt functional integrity of tissues and have pro-inflammatory and tumorigenic properties that act on surrounding cells, termed the senescence-associated secretory phenotype (SASP) [2-7]. Senescent cells have been found to be more prevalent in several tissues of aged mammals *in vivo* [8-12] and the SASP has been implicated in the aging process [13]. Seminal experiments using progeroid and normally aged mice show that health span was markedly improved when senescent cells were cleared from tissues by inducing apoptosis [14-16]. These data indicate a causal link between the presence of senescent cells and the rate of aging.

Several attempts have been made to clarify the role of senescent cells in the aging process of humans. *In vitro,* cells derived from older humans more often show higher expression of markers indicative of senescence compared to cells derived from young humans [17-20]. Senescent cells have also been shown to be more frequently present in tissue sections from older compared to younger humans. Studies in various animal models show that these positive associations between senescence and chronological age markedly differ across various tissues [9;11;21] and may reflect different rates of tissue renewal and or different sensitivity to triggers of senescence.

Here we have conducted a systematic review of the literature reporting on the prevalence of senescent cells dependent on chronological age in various human tissues.

Methods

Selection of studies

A systematic search of the literature was performed on 13-05-2014 in PubMed, EMBASE and Web of Science using the terms "senescence", "tissue"/"biopsy"/"histology", different organ/tissue types and different markers for cellular senescence (see Supplementary methods for complete search strategies) with no limitations on publication dates. The search yielded 6709 articles, of which 2876 were duplicates. A total of 3833 articles were screened on title and abstract by one author (MW); exclusion criteria were (1) animal studies; (2) *in vitro* studies; (3) reviews; (4) conference abstracts; (5) method and theory papers; (6) editorials; and (7) articles in another language than English. Then 458 articles were further evaluated based on

full text by two authors (MW and MS). The same set of exclusion criteria was applied but now included the following additional criteria: (8) the method how senescent cells were detected was not reported; (9) the sole use of telomere length as a marker of senescence; (10) the detection of senescent cells was not related to human tissue; (11) the presence of senescent cells was not linked to neither age or nor disease; (12) the presence of senescent cells was linked to malignant tumors.

The following senescence markers were included: markers of cell-cycle arrest (p16, p21, p53), lack of proliferation markers (Ki67, bmi1), senescence-associated β galactosidase (SAβ-gal), senescence-associated heterochromatic foci (SAHF), DNA damage markers, SASP factors (including cytokines, growth factors, proteases). The lack of or decline of the markers Ki67, bmi1, CD45RA+, CD27+ and CD28+ are considered 'negative' markers of senescence', as the absence of one of these markers is indicative of cellular senescence. Telomere length was excluded as a marker of senescence as determining telomere length in tissue samples is cumbersome [22], sensitivity and specificity of telomere length as a marker of cellular senescence is low [23], and the contribution of telomere attrition to cellular senescence *in vivo* is not established (reviewed in [24;25]).

In 12.2% of the evaluated full text articles evaluators had a different judgment regarding inclusion or exclusion and in 22.1% of the evaluated full text articles one evaluator was undecided regarding inclusion or exclusion. Discrepancies in the judgment between evaluators were solved by including a third author (AM). This resulted in a total of 134 included articles on the association between markers of senescence and chronological age or disease. For this systematic review we further excluded 91 articles because the relation of senescence with chronological age was not reported (only relation with disease reported). The flow diagram of the articles under study is illustrated in Figure 1.

Extraction of data

From each of the 43 included articles the following data were extracted: type and origin of tissue; the number age and gender of the subjects; the marker(s) of senescence and techniques used; the association between the senescence markers and age (primary result), variation of association senescence markers and age, statistical test. If no information on the trend and/or a statistical test was given, raw data points for individual subjects were extracted from figures if possible, in order to calculate the association between senescence markers and age. IBM Statistics SPSS 20 was used for analyses. The direction of association was defined as followed: a positive association is defined as higher senescence across higher age groups (i.e. a higher expression of a positive senescence marker, or a lower expression of a negative marker). A negative association is defined as lower senescence across higher age groups. Associations

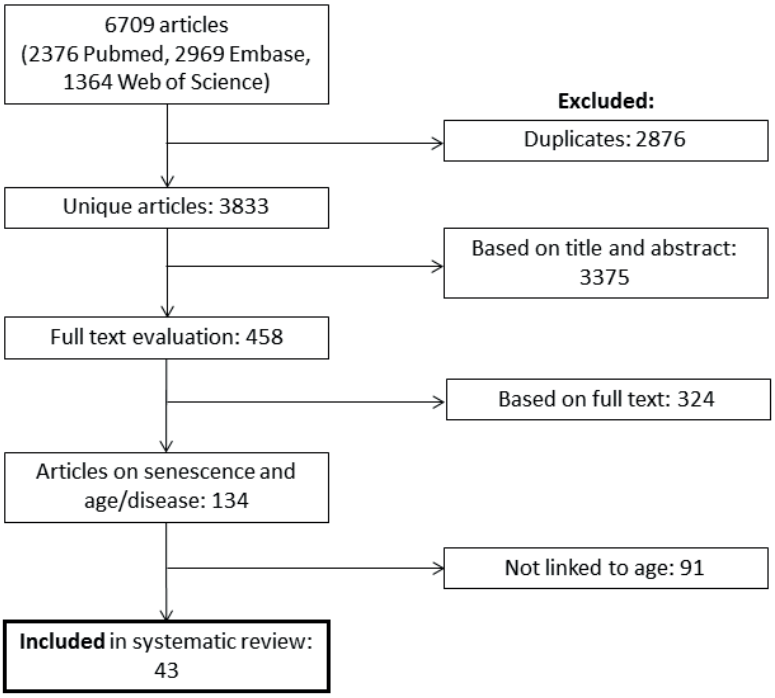

Figure 1. Flow chart of the in- and exclusion of articles.

were further classified by statistical significance. Associations were classified as a statistically significant association at $p<0.05$ and as a trend at $0.05<p<0.10$. All other outcomes were classified as inconclusive ($p>0.10$ or reported as 'ns – not significant' by the source article).

Results

Table 1 depicts the characteristics of the included cohorts (44 cohorts, from 43 articles) arranged by the tissue type that was sampled. Cohorts predominantly used blood (n=10), kidney (n=9) or skin (n=9) samples. Most tissues were acquired perioperatively or post-mortem, resulting in different medical conditions of subjects being studied. The size and the age distribution of the cohorts ranged widely, and only 22 out of 44 study cohorts provided information on gender. Several senescence markers were studied, and in some cohorts multiple markers were used. Among the most frequently used markers were p16 (n=20), SAβ-gal (n=7), p21 (n=5) and p53 (n=5). Most cohorts used either immunohistochemistry (IHC, n=21) or polymerase-chain reaction (PCR, n=10) techniques to detect senescence.

Table 1. Characteristics of included cohorts ordered by tissue type.

Tissue type	Origin of tissue	No. subjects	Age of subjects (yrs)	Gender M/F (%)	Marker	Technique	First author	Year of publication
Artery								
	Internal mammaries, coronary artery bypass surgery	24	M Mean 54.8, SE 5.5; O Mean 77.1, SE 2.2	100/0	p21; cyclin D	WB	Marchand	2011
	Sentinel lymph node biopsy (melanoma-associated profylactic procedure)	61	Y Mean 31.2, SE 1.3; O Mean 70.9, SE 1.4	59/41	p21; SASP (IL-6, IL-8, MCP-1)	PCR	Morgan	2013
Blood								
	Patients undergoing cardiac surgery	44	Y Median 32.8, IQR 28.1-42.1, O Median 69.1, IQR 61.0-74.6	59/41	CD45RA+CD27+/CD4+; CD45RA+CD27+/CD8+	FC	Ferrando-Martinez	2011
	Healthy controls accompanying liver transplant recipients	41	Mean 55.9; SD 13.2	39/61	CD8+CD28+; CD8+CD27+; CD4+CD28+; CD4+CD27+	FC	Gelson	2010
	Heterosexual participants	26	Mean 41; SD 13	81/19	CD45RA+CD31+CD4+; CD45RA+CD31-CD4+		Kilpatrick	2008
	Volunteers undergoing routine physical and medical examination and survivors of an epidemiological study of aging	178	Range 0-93	nr	CD28-CD8+; CD28-CD4+	FC	Lemster [a]	2008
	Healthy donors	36	Range 21-74	nr	CD28-CD57+	FC	Mondal	2013
	Healthy donors	51	Range 18-89	nr	CD28-CD8+	FC	Nociari	1999
	Cohort of breast cancer survivors, part received chemotherapy	176	Median 67; range 50-93	0/100	p16	PCR	Sanoff a	2014
	Prospective adjuvant cohort breast cancer patients, part received chemotherapy	33	Range 32-69	0/100	p16	PCR	Sanoff b	2014
	Healthy donors (no history of inflammatory or malignant disease)	74	Range 24.4-90.1	nr	Ki67	FC	Schonland	2003

Table 1. Characteristics of included cohorts ordered by tissue type. (continued)

Tissue type	Origin of tissue	No. subjects	Age of subjects (yrs)	Gender M/F (%)	Marker	Technique	First author	Year of publication
	Nonsmoking healthy volunteers, <1 hour exercise/week	47	Y Mean 21.8, SD 2.8; O Mean 50.9, SD 7.6	62/38	p53	WB	Werner	2011
Brain								
	Autopsied individuals, postmortem interval range 3 to 43.5 hours, uncertain for fetuses (died in utero)	21	Range fetal-90	40/60	p16	IF	Bhat [a]	2012
Eye								
	Post-mortem	9 (?)	Range <30-75	nr	BMI1; p16	WB; IF; IHC	Abdouh	2012
	Post-mortem, various causes of death	23	Y Median 48.0, IQR 45.5-55.0; O Median 72.5, IQR 72.0-74.0	61/39	SASP (IL1-RA)	IHC	Cao	2013
	Residual corneal tissues obtained after penetrating keratoplasty	27	Range 19-61	nr	p16; p21; p53	PCR	Song	2008
	Death to preservation interval <30 hours. Donor history and condition of tissue did not indicate damaged epithelium	33	Median 37; IQR 24-51	nr	p16	PCR	Wang	2012
Gastro-intestinal								
	Upper gastrointestinal endoscopy	46	Median 69.5; Range 29-90	nr	SAβ-gal	IHC	Going [b]	2002
Heart								
	Post-mortem, no cardiovascular cause of death, clinical/ anatomincal/ histological inclusion criteria provided	74	Range 19-104	57/43	p16	IF	Kajstura	2010
Kidney								

Table 1. Characteristics of included cohorts ordered by tissue type. (continued)

Tissue type	Origin of tissue	No. subjects	Age of subjects (yrs)	Gender M/F (%)	Marker	Technique	First author	Year of publication
	Nephrectomies and renal transplants	58	Range 17.8-93.3	nr	cyclin D	IHC	Berkenkamp	2014
	Nephrectomies and renal transplants	20	Mean 54.4; SD 18; Range 21-80	70/30	p16	IHC	Chkhotua	2003
	Renal transplants	33	Mean 48.0; SD 15.7	45/55	p16	PCR	Gingell-Littlejohn	2013
	Renal transplants	54	Mean 46.3; SD 16.0	69/31	p21	PCR	Koppelstaetter	2008
	Patients with IgA nephropathy	108	Mean 35; SD 11.9	54/46	SAβ-gal; p16; p21	IHC	Liu	2012
	Renal transplants.	73	Mean 46.9; SD 16.3	51/49	p16	PCR	McGlynn	2009
	Nephrectomies, renal transplants and post-mortem	42	Median 51.6; Range 8 weeks-88 years	55/45	p16; p53; p21; SASP (MMP-1, TGFβ1)	PCR	Melk [a]	2004
	Includes diseased kidneys	66	Y Mean 36.4, SD 9.6; M Mean 52.8, SD 20.5; O Mean 47.9, SD 19.5	50/50	p16	IHC	Sis	2007
	Biopsies and nephrectomies	17	Mean 61; SE 2	82/18	p16; SAβ-gal	IHC	Verzola	2008
Lung								
	Lung biopsy samples or surgical resection, judged normal by pathological examination	14	Range 43-82	nr	p16; mH2A	WB	Shivshankar	2011
Prostate								
	Benign tissue from hyperplastic transition zone, from radical prostatectomies	nr	Range 40- >70	100/0	SAβ-gal	IHC	Castro	2003

Table 1. Characteristics of included cohorts ordered by tissue type. (continued)

Tissue type	Origin of tissue	No. subjects	Age of subjects (yrs)	Gender M/F (%)	Marker	Technique	First author	Year of publication
	Specimens from transition zones of radical prostatectomies or transrectal ultrasound-guided biopsies	43	Median 62.0; IQR 57.5-66.0	100/0	SAβ-gal	IHC	Choi	2000
Skin								
	Patients undergoing Moh's micrographic surgery for skin cancer	20	Y Median 35.0, IQR 31.0-37.8; O Median 75.5, IQR 73.0-80.3	55/45	SAβ-gal	IHC	Dimri	1995
	Normal donors, sites of skin biopsies included scalp, eyelid, above the upper lip.	4	Y 1; O >60	nr	Lamin B	IHC	Dreesen [a]	2013
	Punch biopsies from patients undergoing cosmetic or dermatosurgical procedures (facial and abdominal skin)	36	Median 35.1; Range 6-77	50/50	p53	IHC	El-Domyati	2003
	Recruited from department of dermatology	4	Range 18-77	nr	Ki67	IF	Klement	2012
	Skin from circumcision and abdominal punch biopsy	2	Neonatal; 86	nr	p16	IHC	Lee	2010
	Post-chemotherapy	10	Median 18; Range 13-25	80/20	p16; p21	PCR	Marcoux [c]	2013
	Biopsies obtained for medical reasons, from all regions of the body, taken outside of disease-affected areas	33	Range 0-95	nr	p16	IHC	Ressler [a]	2006
	Patients undergoing Moh's micrographic surgery, noncancer tissue samples	53	Range 14-84	64/36	SAβ-gal	IHC	Severino	2000

Table 1. Characteristics of included cohorts ordered by tissue type. (continued)

Tissue type	Origin of tissue	No. subjects	Age of subjects (yrs)	Gender M/F (%)	Marker	Technique	First author	Year of publication
	Patients from department of dermatology, Fitzpatrick skin types I or II, exclusion criteria provided	25	Range 22-89	79/21	53BP1	IHC	Spandau	2012
Testis								
	Testicular tissue removed in surgeries unrelated to testicular germ cell tumours (e.g. prostate cancer, contralateral Leydig tumour), fetal tissue from spontaneous abortions	33	Range fetal-adult	100/0	γH2AX	IHC	Bartkova [a]	2011
Thymus								
	Surgery for reasons other than thymic pathology, no autoimmune disease	20	Range fetal-adult	nr	Ki67; p16	IHC	Kanavaros [a]	2001
Several tissues								
	Obtained from surgeries or autopsies	nr	nr	nr	p16	IHC	Nielsen [d]	1999
	Normal areas and tumor areas	nr	nr	nr	DDR (γH2AX, ATM, 53BP1, CHK2, p53)	IHC	Nuciforo [e]	2007

SD: standard deviation. SE: standard error. IQR: interquartile range. nr: not reported. DDR: DNA damage response. IHC: immunohistochemistry. IF: immunofluorescence. PCR: polymerase chain reaction. FC: flow cytometry. WB: Western blot. Y: young age group. M: middle age group. O: old age group.

[a]: Includes fetal/newborn/infant (≤1 year) tissue.

[b]: 18 subjects without dysplasia or carcinoma

[c]: All donors <25 years.

[d]: Tissue types: brain, spinal cord/peripheral nerves, heart, lung, liver, spleen, kidney, bladder, uterus, ovary, breast, testis, epididymis, prostate, oesophagus, stomach, intestines, pancreas, pituitary, adrenal, thyroid, parathyroid and salivary glands, skin, tonsil, lymph node and bone marrow.

[e]: Mix of normal and tumor tissue. Tissue types: breast, lung, colon-rectum, kidney, larynx, stomach, hematopoietic system, skin, soft tissue, bone.

Table 2 shows the multiple associations (N=78) between senescence and chronological age that were tested in the cohorts. Thirty-four out of the 78 (44%) reported associations showed a positive statistically significant association of cellular senescence and age, and six out of 78 (8%) associations showed a positive trend. Twenty-seven out of 78 (35%) inconclusive associations were reported. One negative statistically significant association of senescence with age was found. Ten out of 78 (13%) associations reported an association with age but provided too little quantification or did not report on a statistical test to solidify their findings.

Table 3 summarizes the associations of senescence with age per tissue. Kidney, blood and skin were used most. Out of 27 associations based on kidney tissue, nine (33%) were positive significant associations, three (11%) were positive trends, and 14 associations (52%) were inconclusive. One (4%) negative significant association of senescence with age was seen with matrix metalloproteinase (MMP-1). The outcomes of the 16 associations in blood samples were differently distributed: nine (56%) were positive significant associations, two (13%) were positive trends, four (25%) associations were inconclusive, and for one association (6%) insufficient information was supplied. In the skin 13 associations were studied, and results were diverse: four (31%) were positive significant associations, four (31%) associations were inconclusive, and insufficient information was supplied for five associations (38%).

Table 4 summarizes the reported associations for the various markers used. The most frequently used marker was p16 with 29 reported associations, though in some cases multiple samples of the same biopsy were stained for p16. With p16 as a marker, 14 (48%) positive significant associations were found, another four (14%) were positive trends, and nine (31%) associations were inconclusive. Two (7%) associations with p16 were reported without sufficient information. SAβ-gal was reported in eight associations, of which four (50%) were inconclusive and for the other four (50%) reported associations insufficient information was supplied. For the marker p21, seven associations were reported: one (14%) was a positive significant association and the other six (86%) associations were inconclusive.

Discussion

We have presented an extensive overview of the associations between senescence markers and chronological age in the literature. Overall, mostly significant positive associations of senescence with chronological age were found in different tissues.

The distribution of reported associations between senescence and chronological age favors positive associations, with only one reported significant negative association. This may be

Table 2. Assocations of senescence markers with age ordered by tissue type.

Tissue type	No. associations tested	Association with age	Specification	Comments	First author	Year of publication
Artery						
	2	±; ±	FC O/M: 1.1 for both p21 and cyclin D		Marchand	2011
	4	+*;+;+*;+*	FC O/Y: 1.9 for p21; 1.7 for IL-6; 7.5 for IL-8; 2.1 for MCP-1		Morgan	2013
Blood						
	2	+*;+*	ΔOY: 13.73% for CD45RA+CD27+/CD4+; 27.82% for CD45RA+CD27+/CD8+	a	Ferrando-Martinez	2011
	4	+;+*;±; ±	r: -0.27 with MFI CD8+CD28+; -0.37 with MFI CD8+CD27+; -0.02 with MFI CD4+CD28+; -0.07 with MFI CD4+CD27+	a	Gelson	2010
	2	+*;±	Slope per year: -0.41% CD45RA+CD31+CD4+; 0.09% CD45RA+CD31-CD4+	a	Kilpatrick	2008
	2	+*;+*	r: 0.618 with % CD28-CD8+; 0.507 with % CD28-CD4+		Lemster	2008
	1	+*	r: 0.729 with %CD28-CD57+		Mondal	2013
	1	+*	ΔOY: 5.45 no. CD28-CD8+		Nociari	1999
	1	+*	Slope: 0.06 log2 expression of p16 per 1 year		Sanoff a	2014
	1	±	ΔOY: -0.23 log2 expression of p16		Sanoff b	2014
	1	nr	Slope: 0.0167 % Ki67 positive per year		Schonland	2003
	1	+*	FC O/Y: 2.16 p53		Werner	2011
Brain						
	1	+*	ΔOY: 43% p16 positive		Bhat	2012
Eye						
	1	+*	ΔOY: 0.56 rel. exp. of bmi1	a	Abdouh	2012
	1	+*	ΔOY: 0.833 stained area of IL-1RA		Cao	2013
	3	+*;±; ±	ΔOY: 0.365 rel.exp. for p16; -0.541 for p21; 0.063 for p53		Song	2008
	1	+*	r: 0.560 with rel. exp. p16		Wang	2012
Gastro-intestinal						

Table 2. (continued)

Tissue type	No. associations tested	Association with age	Specification	Comments	First author	Year of publication
	1	nr	No relation between SAβgal activity and age	b	Going	2002
Heart						
	1	+*	r: 0.83 with % p16 positive		Kajstura	2010
Kidney						
	1	+*	r: 0.27 with % cyclin d positive		Berkenkamp	2014
	6	+;+*;+;+*;+;±	r: 0.299-0.726 with p16	c	Chkhotua	2003
	1	+*	r: 0.597 with rel. exp. p16		Gingell-Littlejohn	2013
	2	±;+*	r: -0.01 with rel. exp. p21; 0.30 with rel. exp. p16		Koppelstaetter	2008
	3	±; ±; ±	r: 0.12 with % SAβgal positive; 0.25 with % p16 positive; 0.11 with % p21 positive		Liu	2012
	1	+*	r: 0.325 with rel. exp. p16		McGlynn	2009
	6	+*;±; ±;–*; ±; ±	Slope rel. exp. per year: 0.016 for p16 (cortex); 0.0003 for p53; 0.0028 for p21; -0.0128 for MMP1; 0.0019 for TGFβ1. R2: 0.03 for rel. exp. p16 (medulla)		Melk	2004
	4	+*;±; ±;+	R^2: 0.044-0.07 with % p16 positive	c, d	Sis	2007
	3	±; ±; ±	r: -0.02 with % p16 positive (glomerulus); 0.02 with % p16 positive (tubule); 0.38 with % SAβgal positive (tubule)		Verzola	2008
Lung						
	2	+*;+*	ΔOY: 2.273 rel. exp. p16; 0.408 rel. exp. mH2A		Shivshankar	2011
Prostate						
	1	nr	ΔOY: 0.31 SAβgal activity		Castro	2003
	1	±	Mean age of SAβgal negative tissue (N=26) 62.0 (SD 7.1) versus mean age of SAβgal positive tissue (N=17) mean age 60.4 (SD 5.9)		Choi	2000
Skin						

Table 2. (continued)

Tissue type	No. associations tested	Association with age	Specification	Comments	First author	Year of publication
	2	nr;nr	Semi-quantative SAβgal positivity per biopsy: Y: 4 -, 3 ±, 2 +, 1 ++ (epidermis); O: 2 ±, 2++, 6 +++ (epidermis); Y: 10 - (dermis); O: 1 -, 4 ++, 5 +++ (dermis)		Dimri	1995
	2	nr;nr	"Robust levels of LMNB1 .. in the majority of young epidermal kerationocytes" " .. in skin from old donors, LMNB1.. were reduced" ".. the number of Ki-67–positive cells in the basal layer was dramatically reduced in aged skin"	a	Dreesen	2013
	2	+*;±	Slope p53 positivity+intensity score per year: 0.06 (facial skin); 0.001 (abdominal skin)		El-Domyati	2003
	1	+*	ΔOY: 13.2 % Ki67 positive	a	Klement	2012
	1	nr	"P16 stained cells were not detected well in Age 1 and Age 10 and numerous p16 stained cells were observed in Age 86"		Lee	2010
	2	±; ±	r: -0.385 with rel. exp. 16; -0.517 with rel. exp. p21		Marcoux	2013
	1	+*	ΔOY: 6 no. p16 positive x staining intensity		Ressler	2006
	1	±	r: 0.09 with no. of SAβgal positive		Severino	2000
	1	+*	ΔOY: 22.32 % 53BP1 positive		Spandau	2012
Testis						
	1	nr	Adult testes: ".. strong staining for γH2AX in spermatocytes.." Foetal testes: "..evident γH2AX staining that is characterized by numerous fine foci"	b	Bartkova	2011
Thymus						
	2	+*;nr	ΔOY: 62.6% Ki67 positive; increase p16 positive cells with age	a, b	Kanavaros	2001
Several tissues						
	several	nr	".. p16 expression in newborns was present only in Hassall's corpuscles, scattered thymic lymphocytes, and rare epithelial cells of the pancreas."	e	Nielsen	1999
	several	±	" .. there is no significant association between individual activated DDR markers and age, .."	b, e	Nuciforo	2007

Legend - table 2. continued
Rel. exp.: relative expression. FC: fold change. Δ: difference. r: correlation coefficient. R^2: coefficient of determination. nr: data not shown/no statistical testing. MFI: mean fluorescence intensity. Y: young age group. M: middle age group. O: old age group.
Direction of association: a positive association is defined as higher senescence (measured with a higher expression of a positive marker, or a lower expression of a negative marker for senescence) at higher ages. A negative association is defined as lower senescence at higher ages.
+*: positive significant association ($p<0.05$). +: positive trend ($0.05<p<0.10$), ±: inconclusive ($p>0.10$ or termed 'ns' in source report). −: negative trend ($0.05<p<0.10$). −*: negative significant association ($p<0.05$).
Comments: a: Negative marker of senescence: absence or low expression expected in senescence. b: Data not shown. c: Measured in several areas of kidney. d: p-value from multivariate model. e: cohort that used several, but less well-defined tissue-markers combinations, therefore unable to count within total number of associations tested.

Table 3. Statistical significance of associations between senescence and chronological age – ordered by type of tissue.

		Significance of associations with age (no., %)					
Tissue	**No.**	**Positive – significant**	**Positive – trend**	**inconclusive**	**Negative – trend**	**Negative – significant**	**Insufficient information reported**
All tissues	78	34 (44)	6 (8)	27 (35)	0 (0)	1 (1)	10 (13)
Kidney	27	9 (33)	3 (11)	14 (52)	0 (0)	1 (4)	0 (0)
Blood	16	9 (56)	2 (13)	4 (25)	0 (0)	0 (0)	1 (6)
Skin	13	4 (31)	0 (0)	4 (31)	0 (0)	0 (0)	5 (38)
Artery	6	3 (50)	1 (17)	2 (33)	0 (0)	0 (0)	0 (0)
Eye	6	4 (67)	0 (0)	2 (33)	0 (0)	0 (0)	0 (0)
Lung	2	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Prostate	2	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	1 (50)
Thymus	2	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)
Brain	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gastro-intestinal	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
Heart	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Testis	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)

No.: number of reported associations. Tissues are sorted on number of reported associations per tissue type. Direction of association: a positive association is defined as higher senescence (measured with a higher expression of a positive marker, or a lower expression of a negative marker for senescence) at higher ages. A negative association is defined as lower senescence at higher ages. Statistically significant: $p<0.05$; a trend: $0.05<p<0.10$; inconclusive: $p>0.10$ or reported as 'ns – not significant' by the source report.

Table 4. Statistical significance of associations between senescence and chronological age – ordered by senescence marker.

		Significance of associations with age (no., %)					
Marker	**No.**	**Positive – significant**	**Positive – trend**	**Inconclusive**	**Negative – trend**	**Negative – significant**	**Insufficient information reported**
All markers	78	34 (44)	6 (8)	27 (35)	0 (0)	1 (1)	10 (13)
p16	29	14 (48)	4 (14)	9 (31)	0 (0)	0 (0)	2 (7)
SAβ-gal	8	0 (0)	0 (0)	4 (50)	0 (0)	0 (0)	4 (50)
Markers naïve T-cells*	8	4 (50)	1 (13)	3 (38)	0 (0)	0 (0)	0 (0)
p21	7	1 (14)	0 (0)	6 (86)	0 (0)	0 (0)	0 (0)
SASP factors*	6	3 (50)	1 (17)	1 (17)	0 (0)	1 (17)	0 (0)
p53	5	2 (40)	0 (0)	3 (60)	0 (0)	0 (0)	0 (0)
Markers differentiated/ senescent T-cells*	4	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ki-67	4	2 (50)	0 (0)	0 (0)	0 (0)	0 (0)	2 (50)
Cyclin D	2	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)
DNA damage markers*	2	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)
Bmi-1	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
LMNB1	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
SAHF	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

N.: number of reported associations. Tissues are sorted on number of reported associations per marker. * Contains a set of markers. Direction of association: a positive association is defined as higher senescence (measured with a higher expression of a positive marker, or a lower expression of a negative marker for senescence) at higher ages. A negative association is defined as lower senescence at higher ages. Statistically significant: $p<0.05$; a trend: $p<0.10$; inconclusive: $p>0.10$ or reported as 'ns – not significant' by the source report.

the result of a real, strong association of senescent cells with chronological age or it may be partly explained by publication bias. Studies with large, significant and expected effects tend to get published more often (positive result bias) and cited more often (citation bias). Studies with small sample sizes are more prone to these types of biases than larger studies as the latter tend to be published and cited irrespective of the outcome [26-28]. Publication bias occurs in all scientific fields, and biogerontological research is no exemption. For example, when replicative capacity of fibroblasts *in vitro* was related dependent on the chronological age of the donor, the earliest but smaller studies predominantly found negative significant associations whereas more recent and larger studies did not confirm this association [29]. In the same area, a meta-analysis on the relation between DNA damage and age in animal tissue also provided arguments that publication and citation bias is at play [30].

The presence of senescent cells at older age has been postulated to be a result of an ongoing induction of this cellular phenotype via the exposure of various triggers (e.g. increased DNA damage or oncogenic signaling, reviewed in [31;32]), or indirectly via induction of senescence in neighboring cells of senescent cells [33]. An alternative interpretation of the presence is a reduced clearance of senescent cells. Some mouse studies have implicated the role of immune cells in the removal of senescent cells in premalignant lesions [34;35]. The precise mechanisms how senescent cell are cleared in non-malignant tissue have yet to be established, but an impaired balance between generation and clearance of senescent cells provides an another plausible hypothesis for the positive associations of senescence and chronological age. Only one negative significant association in this systematic review was found, namely a significant decrease of MMP-1 with age in the kidney. Although SASP is diverse, most SASP factors including MMP-1 are upregulated in senescent fibroblasts [36].

The different patterns of the associations in the various tissues might be the result of biological differences, such as tissue renewal rate or sensitivity to senescence triggers. The cell turnover rate of tissue varies widely[37;38]. Moreover homeostasis of the various tissues might be differently affected during aging [39]. Tissue-specificity of (unknown) environmental exposures are another explanation for the findings. Mice exposed to cigarette smoke show locally increased senescence in the nasal epithelium, whereas UV exposure induces senescence in the skin [40], indicating that environmental triggers can affect specific tissues more extensively than others. To the best of our knowledge information is lacking in humans on whether associations between senescence and age vary throughout the body, or whether they are linked intra-individually. Future studies on cellular senescence in several tissue types of the same individual should help to shed light on this question.

The various markers show different patterns of associations with age, and this is most prominent for the expression patterns of p16 and SAβ-gal. Associations using p16 are more frequently positive significant whereas reported associations with SAβ-gal were more often inconclusive. Moreover, for more than half of the associations sufficient information on statistical testing was missing. This might be explained by the fact that SAβ-gal was the marker of choice in the initial (more explorative) studies into senescence in human tissue [41], and results were more often not or semi-quantified compared to later studies. Taken together, the available senescence markers all have their caveats and restrictions in their use; and there is not yet an universal marker of senescent cells, which was addressed in a recent review [23].

Among the strengths of this study are the broad search strategy designed to capture many articles and extensive evaluation of articles for inclusion in the systematic review. We did

not exclude any tissues and thus provide an overview of senescence in human aging in different organs. There are some limitations of this systematic review. First, while we aimed to design an inclusive broad search strategy, some studies might have still been missed. For example, we did not specifically add markers of immunosenescence in the search strategy, which might have led to an underrepresentation of those studies. Second, we only included articles in English, possibly biasing the results. Lastly we did not exclude any age ranges, thus associations between senescent cells with age also include data from foetuses to young adults. The associations between senescent cells with age might be different in the development phase versus reproductive and post-reproductive phases of life.

In conclusion, mainly positive associations of senescence with chronological age were found, which could be a real effect but can also to be due to an influence of publication bias. On a critical note, studies should more precisely report on the strength and variances of the associations using appropriate statistical testing, and to better describe the characteristics of patients/subjects from whom the tissues were sampled. This quantification can aid future systematic reviews and meta-analysis in the field and help to fairly judge the relevance of cellular senescence to the aging process in humans.

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Search strategy

PubMed

("senescent"[all fields] OR "senescence"[all fields]) AND ("Tissues"[Mesh] OR "tissues"[all fields] OR "tissue"[all fields] OR "cell"[tiab] OR "cells"[tiab] OR "Biopsy"[Mesh] OR "Biopsy"[all fields] OR "Autopsy"[Mesh] OR "Autopsy"[all fields] OR "Histology"[Mesh] OR "Histology"[all fields] OR "histologic"[all fields]) AND ("Brain"[Mesh] OR "brain"[tiab] OR "Eye"[Mesh] OR "eye"[tiab] OR "eyes"[tiab] OR "cornea"[tiab] OR "lens"[tiab] OR "retina"[tiab] OR "retinal"[tiab] OR "Endocrine Glands"[Mesh] OR "thyroid"[tiab] OR "parathyroid"[tiab] OR "Cardiovascular System"[Mesh] OR "heart"[tiab] OR "myocard"[tiab] OR "myocardial"[tiab] OR "cardiovascular"[tiab] OR "artery"[tiab] OR "arteries"[tiab] OR "arterial"[tiab] OR "vein"[tiab] OR "venous"[tiab] OR "endothelial"[tiab] OR "Skin"[Mesh] OR "skin"[tiab] OR "dermis"[tiab] OR "dermal"[tiab] OR "epidermis"[tiab] OR "epidermal" OR "adipose"[tiab] OR "Lung"[Mesh] OR "lung"[tiab] OR "Lymphoid Tissue"[Mesh] OR "thymus"[tiab] OR "Breast"[Mesh] OR "breast"[tiab] OR "mammae"[tiab] OR "Digestive System"[Mesh] OR "intestinal"[tiab] OR "esophagus"[tiab] OR "esophagal"[tiab] OR "gastric"[tiab] OR "liver"[tiab] OR "pancreas"[tiab] OR "pancreatic"[tiab] OR "Urogenital System"[Mesh] OR "bladder"[tiab] OR "kidney"[tiab] OR "renal"[tiab] OR "adrenal"[tiab] OR "spleen"[tiab] OR "prostate"[tiab] OR "prostatic"[tiab] OR "uterus"[tiab] OR "uterine"[tiab] OR "ovaries"[tiab] OR "ovarian"[tiab] OR "testes"[tiab] OR "testicular"[tiab] OR "Muscles"[Mesh] OR "muscle"[tiab] OR "muscular"[tiab]) AND ("p16INK4a"[all fields] OR "Cyclin-Dependent Kinase Inhibitor p16"[Mesh] OR "Cyclin-Dependent Kinase Inhibitor p21"[Mesh] OR "p21"[all fields] OR "p21cip1"[all fields] OR "p21:MIB-1"[all fields] OR "p21WAF1/Cip"[all fields] OR "Tumor Suppressor Protein p53"[Mesh] OR "p53"[all fields] OR "GLB1 protein, human" [Supplementary Concept] OR "beta-galactosidase"[all fields] OR "SA-βgal"[all fields] OR "SA-betagal"[all fields] OR "H2AFX protein, human" [Supplementary Concept] OR "γH2AX"[all fields] OR "phospho-Histone H2A.X"[all fields] OR "γH2A.X"[all fields] OR "senescence-associated secretory phenotype"[all fields] OR "SASP"[all fields] OR "Ki-67 Antigen"[Mesh] OR "proliferation arrest"[all fields] OR "Ki-67"[all fields] OR "phospho-checkpoint 2 kinase"[all fields] OR "phospho-Chk2"[all fields] OR "Chromatin Assembly and Disassembly"[Mesh] OR "chromatin remodelling"[all fields] OR " senescence associated heterochromatic foci"[all fields] OR "senescence associated heterochromatic foci"[all fields] OR "SAHF"[all fields] OR "DNA damage response"[all fields] OR "DDR"[tiab] OR "DNA damage foci"[all fields] OR "DNA damage focus"[all fields] OR "Telomere"[Mesh] OR "Telomere Shortening"[Mesh] OR "Telomere Homeostasis"[Mesh] OR "telomere"[all fields] OR "telomeres"[all fields])

EMBASE

("senescent".ti,ab. OR "senescence".ti,ab.) AND (exp *tissues/ OR "tissues".ti,ab. OR "tissue".ti,ab. OR "cell".ti,ab. OR "cells".ti,ab. OR exp *biopsy/ OR "Biopsy".ti,ab. OR *autopsy/ OR "Autopsy".ti,ab. OR exp *histology/ OR "Histology".ti,ab. OR "histologic".ti,ab.) AND (exp *Brain/ OR "brain".ti,ab. OR exp *Eye/ OR "eye".ti,ab. OR "eyes".ti,ab. OR "cornea".ti,ab. OR "lens".ti,ab. OR "retina".ti,ab. OR "retinal".ti,ab. OR exp *endocrine system/ OR "thyroid".ti,ab. OR "parathyroid".ti,ab. OR exp *Cardiovascular System/ OR "heart".ti,ab. OR "myocard".ti,ab. OR "myocardial".ti,ab. OR "cardiovascular".ti,ab. OR "artery".ti,ab. OR "arteries".ti,ab. OR "arterial".ti,ab. OR "vein".ti,ab. OR "venous".ti,ab. OR "endothelial".ti,ab. OR exp *Skin/ OR "skin".ti,ab. OR "dermis".ti,ab. OR "dermal".ti,ab. OR "epidermis".ti,ab. OR "epidermal" OR "adipose".ti,ab. OR exp *Lung/ OR "lung".ti,ab. OR exp *Lymphoid Tissue/ OR "thymus".ti,ab. OR exp *Breast/ OR "breast".ti,ab. OR "mammae".ti,ab. OR exp *Digestive System/ OR "intestinal".ti,ab. OR "esophagus".ti,ab. OR "esophagal".ti,ab. OR "gastric".ti,ab. OR "liver".ti,ab. OR "pancreas".ti,ab. OR "pancreatic".ti,ab. OR exp *Urogenital System/ OR "bladder".ti,ab. OR "kidney".ti,ab. OR "renal".ti,ab. OR "adrenal".ti,ab. OR "spleen".ti,ab. OR "prostate".ti,ab. OR "prostatic".ti,ab. OR "uterus".ti,ab. OR "uterine".ti,ab. OR "ovaries".ti,ab. OR "ovarian".ti,ab. OR "testes".ti,ab. OR "testicular".ti,ab. OR exp *Muscle/ OR "muscle".ti,ab. OR "muscular".ti,ab.) AND ("p16INK4a".ti,ab. OR *cyclin dependent kinase inhibitor 2A/ OR "Cyclin-Dependent Kinase Inhibitor p16".ti,ab. OR *cyclin dependent kinase inhibitor 1A/ OR "p21".ti,ab. OR "p21cip1".ti,ab. OR "p21:MIB-1".ti,ab. OR "p21WAF1/Cip".ti,ab. OR Protein p53/ OR

"p53".ti,ab. OR *beta galactosidase/ OR "beta-galactosidase".ti,ab. OR "SA-βgal".ti,ab. OR "SA-betagal".ti,ab. OR "γH2AX".ti,ab. OR "phospho-Histone H2A.X".ti,ab. OR "γH2A.X".ti,ab. OR "senescence-associated secretory phenotype".ti,ab. OR "SASP".ti,ab. OR *Ki-67 Antigen/ OR "proliferation arrest".ti,ab. OR "Ki-67".ti,ab. OR "phospho-checkpoint 2 kinase".ti,ab. OR "phospho-Chk2".ti,ab. OR exp *"Chromatin Assembly and Disassembly"/ OR "chromatin remodelling".ti,ab. OR "senescence associated heterochromatic foci".ti,ab. OR "senescence associated heterochromatic foci".ti,ab. OR "SAHF".ti,ab. OR "DNA damage response".ti,ab. OR "DDR".ti,ab OR "DNA damage foci".ti,ab. OR "DNA damage focus".ti,ab. OR *Telomere/ OR *Telomere Shortening/ OR *Telomere Homeostasis/ OR "telomere".ti,ab. OR "telomeres".ti,ab.)

Web of Science

TS= (senescent OR senescence) AND TS=(tissues OR tissue OR cell OR cells OR Biopsy OR Autopsy OR Histology OR histologic) AND TI=(brain OR eye OR eyes OR cornea OR lens OR retina OR retinal OR thyroid OR parathyroid OR heart OR myocard OR myocardial OR cardiovascular OR artery OR arteries OR arterial OR vein OR venous OR endothelial OR skin OR dermis OR dermal OR epidermis OR epidermal OR adipose OR lung OR Lymphoid OR thymus OR breast OR mammae OR intestinal OR esophagus OR esophagal OR gastric OR liver OR pancreas OR pancreatic OR bladder OR kidney OR renal OR adrenal OR spleen OR prostate OR prostatic OR uterus OR uterine OR ovaries OR ovarian OR testes OR testicular OR muscle OR muscular) AND TS= (p16INK4a OR cyclin dependent kinase inhibitor 2A OR Cyclin-Dependent Kinase Inhibitor p16 OR cyclin dependent kinase inhibitor 1A OR p21 OR p21cip1 OR p21:MIB-1 OR p21WAF1/Cip OR p53 OR beta-galactosidase OR SA-βgal OR SA-betagal OR γH2AX OR phospho-Histone H2A.X OR γH2A.X OR senescence-associated secretory phenotype OR SASP OR proliferation arrest OR Ki-67 OR phospho-checkpoint 2 kinase OR phospho-Chk2 OR chromatin remodelling OR senescence associated heterochromatic OR SAHF OR DNA damage response OR DDR OR DNA damage foci OR DNA damage focus OR telomere OR telomeres)

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