Microarray analysis of replicative senescence

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Background: Limited replicative capacity is a defining characteristic of most normal human cells and culminates in senescence, an arrested state in which cells remain viable but display an altered pattern of gene and protein expression. To survey widely the alterations in gene expression, we have developed a DNA microarray analysis system that contains genes previously reported to be involved in aging, as well as those involved in many of the major biochemical signaling pathways.

Results: Senescence-associated gene expression was assessed in three cell types: dermal fibroblasts, retinal pigment epithelial cells, and vascular endothelial cells. Fibroblasts demonstrated a strong inflammatory-type response, but shared limited overlap in senescent gene expression patterns with the other two cell types. The characteristics of the senescence response were highly cell-type specific. A comparison of early- and late-passage cells stimulated with serum showed specific deficits in the early and mid G1 response of senescent cells. Several genes that are constitutively overexpressed in senescent fibroblasts are regulated during the cell cycle in early-passage cells, suggesting that senescent cells are locked in an activated state that mimics the early remodeling phase of wound repair.

Conclusions: Replicative senescence triggers mRNA expression patterns that vary widely and cell lineage strongly influences these patterns. In fibroblasts, the senescent state mimics inflammatory wound repair processes and, as such, senescent cells may contribute to chronic wound pathologies.

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Background

Historically, cellular senescence has been defined as the terminal phase of passaged primary human cell populations, a response more accurately defined as replicative senescence. It is now recognized, however, that a similar phenotype can be achieved in both normal and transformed cells by a variety of challenges, such as oxidative stress [1,2], radiation [3], activated oncoproteins and kinases [4–6], cyclin-dependent kinase (CDK) inhibitors [7] and others [8], leading to the concept that cellular senescence represents a fate choice that can be influenced by both pro- and anti-mitogenic stimuli. Senescent cells are not stimulated to divide by serum or passage in culture, and senescence invokes a specific cell-cycle profile that differs from most damage-induced arrest processes or contact inhibition [9]. An enlarged cell size, expression of a pH-dependent β-galactosidase activity [10] and an altered pattern of gene expression [11,12] further mark senescent cells.

Serial cultivation of normal cell strains results in the shortening of telomeres [13] and, in many strains, this process can be prevented by the expression of hTERT, the catalytic protein component of telomerase [14,15]. To understand the baseline changes in gene expression at senescence, we have used cDNA microarrays to study senescence-induced gene expression patterns in different cell types. The cell types examined here — dermal fibroblasts, retinal pigment epithelial cells and vascular endothelial cells — represent biologically diverse lineages that senesce with similar gross phenotypes. The invoked gene expression patterns differ greatly in a cell-type specific manner, however, suggesting that many of the observed alterations result from collateral activation of pathways that are set by cell lineage.

Results

Expression profiles of cell strains at replicative senescence

The three human cell strains chosen were dermal fibroblasts (BJ), retinal pigment epithelial cells (RPE340) and vascular endothelial cells (HUVECs). The replicative capacity of these cells varies, and senescence in all three strains is accompanied by a shortening of mean terminal restriction fragment (TRF) lengths, significant β-galactosidase activity at pH 6.0 (senescence-associated β-galactosidase [10]) and a characteristic cell-cycle distribution, including low numbers of cells in S phase and high numbers in G1 and G2 [9] (Figure 1). In addition, BJ and

Figure 1

	BJ	RPE340	HUVEC
PDL	93.0	53.5	49.5
TRF (kbp)	6.3	4.3	6.1
%G1	71.4	81.8	84.5
%S	4.5	1.9	3.9
%G2	24.1	16.2	9.8

Current Biology

Analyses of senescent primary human cell lines. Photomicrographs are of senescent cultures stained for senescence-associated β-galactosidase (SA-βGal) [10]. Population doubling levels (PDLs) for senescent cultures are indicated, as well as telomere length (mean TRF in kilobase pairs (kbp)), and cell-cycle stage distribution as determined by multicycle FACS analysis.

RPE340 [14] and HUVEC (E.C., unpublished observations) can be immortalized by the expression of telomerase from an hTERT transgene. By these criteria, all three strains of cells undergo a similar arrest process — replicative senescence — initiated by a common effector, the critical shortening of telomeres.

Genes showing at least a 2.5-fold differential in expression in senescent and early-passage cells in multiple independent experiments are listed in Figure 2. Compared with quiescent early-passage cells, BJ fibroblasts at senescence have higher levels of the matrix-regulating proteins stromelysin-1 and stromelysin-2, plasminogen activator inhibitors PAI-1 and PAI-2, and urokinase plasminogen activator (uPA) as well as the inflammatory regulators monocyte chemotactic protein-1 (MCP-1), Gro-α, and interleukin-15 (IL-15). Conversely, senescent fibroblasts significantly underexpressed mRNAs for prostaglandin-1 synthase, elastin, stromelysin-3 and other proteins.

In high-serum conditions, genes that participate in cell division (for example those for cyclin A, cyclin B1, Cdc20, Cdc2, thymidine kinase and thymidylate synthase) are clearly expressed at higher levels in early-passage fibroblasts. Under these conditions, genes for secreted collagens Iα1 and IIIα1, elastin and cytokeratin II type 7 are also more highly expressed in early passage cells.

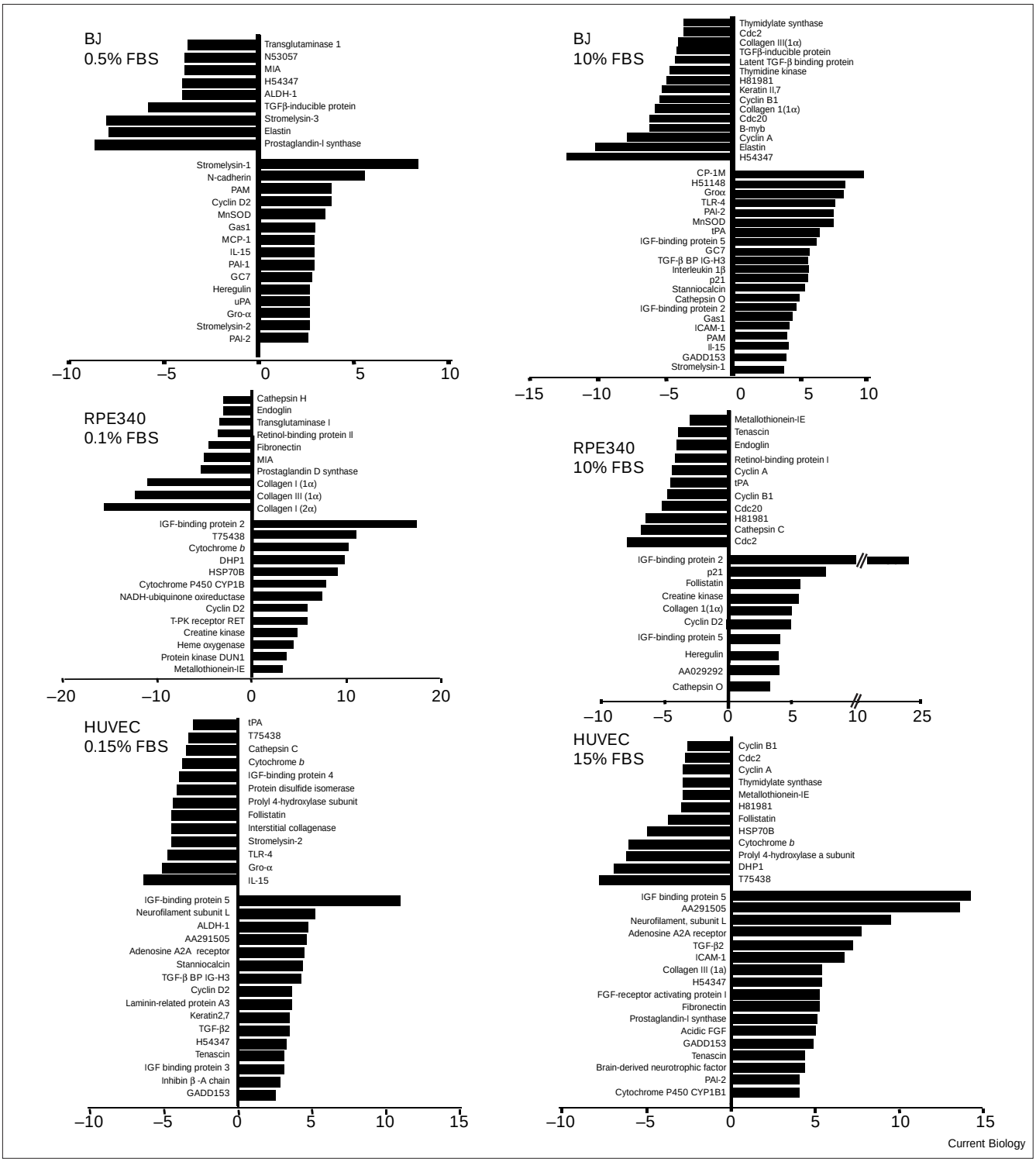
Senescent BJ fibroblasts in high serum express inflammation-associated genes at still higher levels than in low serum, including the chemokines MCP-1 and Gro-α, cytokines IL-15 and IL-1β, the interleukin-1 (IL-1) receptor homolog Toll protein (Tlr-4) and intercellular adhesion molecule-1 (ICAM-1). The CDK inhibitor p21 is overexpressed, as is the growth-arrest-specific protein 1 (Gas1) and growth-arrest and DNA-damage inducible protein 153 (GADD153). In addition, levels of mRNA for insulin-like growth factor binding proteins 2 and 5 (IGF-BP2, IGF-BP5), stanniocalcin and cathepsin O were elevated, and levels of peptidyl-α amidating monooxygenase (PAM), a prohormone-converting enzyme [16], remained high regardless of serum conditions.

The expression patterns observed in the other two cell types differed significantly from that of the dermal fibroblasts. In RPE340 cells, the differences in expression of mRNA for IGF-BP2, in both high and low serum, and a notable inability of the senescent cells to express a series of collagens in low-serum conditions were most obvious. Follistatin, a potential activin antagonist protein [17], was significantly expressed in senescent RPE340s whereas senescence repressed the expression of prostaglandin D synthase, a potential retinoid-binding protein [18], and cellular retinol-binding protein-1. Unlike BJ cells, senescence in RPE340s does not result in a significant increase in the expression of inflammatory chemokines or cytokines.

HUVEC cells display a dramatically different pattern of gene expression, in which many of the markers of senescence in fibroblasts are expressed in an inverse fashion. Inflammatory and immune-response genes such as those for IL-15 and Toll protein are all repressed in senescent HUVECs at low serum, as are the matrix proteases. Senescence in HUVECs induces the expression of IGF-BP5, neurofilament subunit L, transforming growth factor-β2 (TGFβ2) and adenosine A2A receptor, regardless of serum conditions. In high-serum conditions, ICAM-1 and GADD153 are overexpressed at senescence, as in fibroblasts.

To assess the extent that senescence-associated patterns of gene expression are preserved among cell strains of similar type, we examined two additional dermal fibroblast lines. C4 and MA were derived from the hand and ankle respectively of the same donor and are thus genetically identical. In general, patterns of gene expression in these two lines varied from that of BJ fibroblasts, though some shared trends are apparent. The expression of matrix proteases and inflammatory chemokines and cytokines at senescence is common to all the dermal fibroblast lines, though the set of expressed markers varies (Table 1a). A comparison of the MA and C4 strains suggests that the magnitude of the response at senescence and the particular genes induced varies even in genetically identical cells derived from the same tissue type. We also examined additional unrelated RPE strains, 338 and 341, and observed similar trends to those in RPE340 at senescence: an upregulation of IGF-BP2 and follistatin mRNAs, and a significant decrease in the expression of collagens, cytokeratins, and associated factors such as transglutaminase-1 and thrombospondin-2 (Table 1b).

Figure 2



Differential gene expression at senescence. Cell strains BJ, RPE340 and HUVEC were grown to senescence and held in high or low fetal bovine serum (FBS) as described in Materials and methods. Genes listed had expression ratios of > 2.4-fold in two or more independent array analyses. Genes expressed predominantly in early-passage cultures were given negative values; genes expressed predominantly in late-passage cultures were given positive values. Expressed sequence tag identifiers are used for re-sequenced clones that failed to match the intended target gene. See text and Supplementary material for abbreviations.

Table 1

(a) Pattern of gene expression in dermal fibroblasts at senescence.

	BJ		C4		MA	
%FBS	0.5	10	0.5	10	0.5	10
Stromelysin-1	8.2	4.1	1.0	22.0	–1.5	1.1
Stromelysin-2	2.6	2.0	1.6	5.9	1.9	1.6
Collagenase	2.0	1.7	–1.4	7.6	2.6	1.5
tPA	1.7	6.3	5.5	1.9	2.5	8.1
uPA	2.7	3.4	1.7	4.8	–1.2	1.2
MCP-1	2.9	9.0	2.7	4.3	2.5	3.4
Gro-α	2.8	6.6	1.6	5.8	2.5	1.2
IL-15	2.9	4.1	2.5	9.7	4.0	2.6
IL-1β	1.7	5.6	–1.2	3.1	1.3	1.2
Cathepsin O	2.9	4.8	4.2	3.9	2.2	4.2
Elastin	–8.4	–5.5	–4.5	–4.5	–8.3	–13.9
MnSOD	3.4	7.4	2.9	2.6	3.8	1.6
Tlr-4	2.0	7.7	1.5	7.3	1.9	1.6
ICAM-1	–1.2	4.8	1.2	3.7	5.4	3.7

(b) Pattern of gene expression in retinal pigment epithelial cells at senescence.

	RPE340		RPE338		RPE341	
%FBS	0.1	10	0.1	10	0.1	10
IGF-binding protein-2	15.3	22.0	6.0	6.9	1.8	4.1
Collagen Iα2	–16.0	1.1	–10.6	–3.6	–58.0	–18.6
Collagen Iα1	–9.5	3.4	1.9	1.4	–26.5	–8.2
Collagen IIIα1	–9.8	1.4	1.9	3.3	–16.5	–4.0
Keratin I,18	1.4	–2.6	–3.8	–2.6	–8.1	–10.6
Keratin II,7	–1.0	–2.4	–18.5	–23.7	–24.8	–23.2
Transglutaminase 1	–3.5	–4.0	1.8	–1.9	–5.2	–3.0
Creatine kinase	3.6	7.3	3.9	2.3	1.6	2.8
Follistatin	–1.6	5.8	4.7	6.0	1.5	3.1
Cathepsin O	–2.1	3.0	2.5	2.7	24.3	11.7
Ceruloplasmin	1.4	1.7	1.0	3.7	14.6	7.0
Retinol-binding protein-1	–3.1	–3.2	1.2	1.2	–3.2	–4.4
Stanniocalcin	1.2	1.3	5.6	9.9	2.3	5.7
Thrombospondin 2	1.2	–3.5	–7.0	–3.3	–10.7	–16.8

RNA was prepared from three independent (a) dermal fibroblast or (b) RPE strains maintained in high or low serum. Positive numbers indicate fold-overexpression in senescent cultures; negative numbers indicate fold-overexpression in early passage cultures. See text and Supplementary material for abbreviations.

Time course of the response to serum stimulation

We compared the responses of early- and late-passage BJ fibroblasts to serum stimulation and a complete listing of responsive genes is provided as Supplementary material. In early-passage cells, late G1/early S-phase markers, such as Cdc2, cyclin B1, proliferating cell nuclear antigen, thymidylate synthase, thymidine kinase, Wee1 and DNA polymerase γ were induced between the 8 and 24 hour time points and were weakly induced or absent in senescent cells (see Supplementary material). The induction of c-*fos* mRNA was apparent within 30 minutes of serum stimulation in early-passage cultures and peaked at 1 hour, whereas in senescent cultures the response was attenuated (Figure 3a). Similarly, mRNAs for IκBα, tristetraproline (TTP), JunB and early growth response protein 1 (EGR-1) are all induced within 1 hour of stimulation in early-passage cells, whereas they are only weakly induced in senescent fibroblasts. The induction of mRNAs for connective tissue growth factor (CTGF) and mitogen-activated protein (MAP) kinase phosphatase 1 appeared unperturbed in senescent cells, indicating that substantial aspects of the early response to serum are maintained. Conversely, there was a pronounced induction of Id-2 in senescent cultures.

Several genes that are serum responsive in early-passage fibroblasts are constitutively upregulated in senescent cells. In early-passage cells, mRNAs for stromelysin-1, MCP-1, PAI-1, PAI-2 and others are induced by serum (Figure 3b). Conversely, collagen transcript levels decrease substantially following serum addition. The expression of many of the serum-inducible genes peaks near the 8 hour time point, at which time the cells would be in transit through the G1/S boundary.

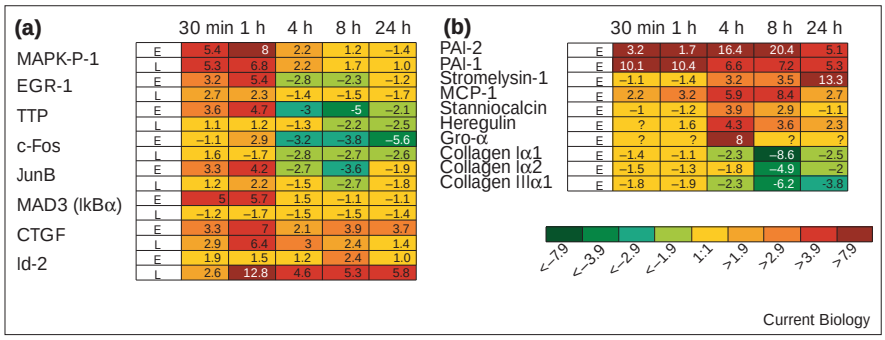
Discussion

Senescence-associated gene expression

All three cell strains reported here undergo an arrest characterized by a distinctive cell-cycle distribution, changes in cell morphology and the shortening of telomeres. Senescent cells have a cell-cycle distribution that is clearly different from that of quiescent cells, as a substantial population of cells with G2 DNA content persists [9] (Figure 2). In high-serum conditions, this arrest includes expression of the CDK inhibitor p21, and growth arrest specific protein (Gas1) in BJ fibroblasts and RPE340 strains, but not in HUVECs. The overexpression of cyclin D2 was observed in all three cell strains and may reflect a common cell-cycle braking mechanism [19,20]. Interestingly, in none of the cell strains examined did we observe an upregulation of mRNA for the CDK inhibitor p16, as reported for other cell strains [21,22], and RT–PCR analysis of mRNA from senescent BJ fibroblasts confirmed the lack of induction of mRNA for p16 (data not shown). The induction of p21 at senescence has been reported to be transient in some cell lines, whereas the induction of p16 can occur after prolonged arrest [23], and this may also account for our observed results with different cell lines. The data presented here strongly suggest that even with a common initiating signal — shortened telomeres — the ensuing arrest may be triggered by different pathways that are set by the specific cell type, yet result in a similar distribution of cells in the G1 and G2 phases of the cell cycle.

In fibroblasts, the expression of matrix-degrading proteases and inflammatory chemokines and cytokines is a consistent trend at senescence and, in this respect, the

Figure 3



Time course of serum stimulation. (a) Early passage (E: PD30) or late passage (L: PD89) BJ cultures were held in 0.5% serum for 2 days, then stimulated with 10% FBS. RNA levels from cultures at the indicated time points (Cy5 channel) were compared with the uninduced starting culture (Cy3 channel). Positive values indicate higher expression in induced cells; negative values indicate lower expression in induced cells. Question marks indicate that there was insufficient signal for detection. A complete listing of serum-responsive genes from this analysis is provided in Supplementary material. (b) The serum-responsiveness of select senescence-regulated genes in early passage (PD30) BJ fibroblasts.

senescence response appears to overlap substantially with gene expression patterns observed in activated fibroblasts during wound healing [24–26]. MCP-1, Gro-α, IL-1β and IL-15 are strong effectors of macrophage and neutrophil recruitment and activation [27,28]. The upregulation of Toll (Tlr-4) in senescent fibroblasts confirms the overall immune response behavior at senescence. Tlr-4 is an IL-1 receptor homolog and is implicated in the activation of the gene regulatory protein NF-κB, a function proposed to be part of the innate immune response [29]. The induction of IL-15 at senescence is also consistent with an innate immune response, as IL-15 can be induced by NF-κB-dependent transcription [30] and also participates in inflammatory disease processes [28].

Deficiencies in the response of senescent cells to serum stimulation have been reported, and include an inability to induce the expression of c-*fos* mRNA [31] and markers of late G1 and S phase [32]. In response to serum, expression of inflammatory chemokines, matrix-degrading proteases and their modulators is induced in early-passage dermal fibroblasts, and expression of matrix collagens is reduced. This transient burst of activity may represent the natural response of these cells in wound repair [24]. Id-2 transcripts were hyper-induced in serum-stimulated senescent fibroblasts, which may be linked to the ability of cytokines to induce Id-2 expression [33]. The levels of mRNAs for many of these induced genes peak at a period predicted to span the G1/S boundary ([24] and this study); thus, the senescence response may mimic an activated state that would inappropriately continue to recruit immune responses, disrupt matrix repair and prevent the completion of normal wound repair process. The induction of matrix proteases in skin exposed to ultraviolet radiation [34] also involves the activation of the MAP kinase signaling cascade [35], a signaling cascade that can also induce a senescence-like phenotype in fibroblasts [4–6]. The gene expression patterns in many of these senescence-like states overlap substantially with those in telomere-induced senescence (W.F., D.N.S., R. Allsopp, S. Lowe, and G. Ferbeyre, unpublished observations) and thus are likely to use many of the same activation processes.

The pattern of gene expression at senescence varies substantially in different cell types. Although the expression of matrix and structural proteins, such as the collagens, keratins and auxiliary factors, is repressed in RPE cells, inflammatory regulators are not induced, in contrast to dermal fibroblasts. Physiologically, this would make sense, as an acute inflammatory response in a tissue critical for normal vision would be likely to have deleterious consequences. However, as the RPE layer has a central role in the deposition and maintenance of extracellular matrix in the retina, decrements in the ability of senescent RPE cells to maintain appropriate expression patterns, as evidenced by decreased expression of collagens, keratins, aggrecan, transglutaminase and so on, would be predicted to have adverse affects on retinal architecture. Dysfunction of the RPE cell layer is considered to be a substantial factor in the development of age-related macular degeneration [36].

Surprisingly, early-passage HUVECs overexpress many of the markers associated with senescence in dermal fibroblasts, such the pro-inflammatory molecules IL-15 and Tlr-4. In very low serum, vascular endothelial cells are often induced to undergo apoptosis, and the conditions used in this study (0.15% fetal bovine serum (FBS)) have been set to induce cessation of growth (quiescence) while preventing apoptosis. NF-κB-mediated activities have been shown to prevent the induction of apoptosis and to promote survival in endothelial cells [37,38]. As senescent cells have been reported to be resistant to apoptosis [39], apoptosis and senescence may be alternative cell fates available to cells under stress. Such dramatic inversions of expression patterns in different cell types strongly suggest that the most obvious effects of senescence are played out

through pathways determined by the cells' differentiated state, presumably as a result of the activation of alternative signaling circuitries set during development. Substantial variation in expression patterns is even evident in isogenic matched dermal fibroblast lines such as C4 and MA.

Conclusions

The patterns of gene expression observed at senescence are strongly influenced by cell lineage. Even with a common initiating event, such as the critical shortening of telomeres, the accompanying arrest profile is highly specific for cell type and strain. The strong inflammatory response observed in dermal fibroblasts mimics expression patterns seen in serum-stimulated cells and in wound repair, suggesting that senescent fibroblasts are locked in an activated state that may exacerbate chronic wound healing processes.

Materials and methods

Cell culture

BJ dermal fibroblasts were a kind gift from J. Smith (Baylor College of Medicine, Houston) and human umbilical vein endothelial cells (HUVEC) were a kind gift from T. Maciag (American Red Cross). RPE340, RPE338 and RPE341 cells were derived from retinal specimens (L. Hjelmland, University of California at Davis). C4 and MA dermal fetal fibroblasts were derived at Geron from hand and medial ankle skin samples respectively from the same human donor. Diploid human cell strains were cultured in humidified chambers with 5% CO_2 (BJ, C4, MA, HUVEC) or 10% CO_2 (RPE340, 338, 341) and passaged serially until the observed S-phase fraction, determined by multicycle analysis by fluorescence-activated cell sorting (FACS), was reduced to <5%. Fibroblast and RPE cells were grown directly on plastic culture dishes, HUVEC were grown on gelatin-coated culture dishes. See Supplementary material for a complete listing of the passage history of these cultures. Early- and late-passage fibroblasts were held in 10% fetal bovine serum (FBS) or held in 0.5% FBS in subconfluent conditions for 5 days before harvest. RPE cells were held in 10% or 0.1% FBS under confluent conditions for 10 days. HUVEC were held in 15% or 0.15% FBS under subconfluent conditions.

Analyses

Cells were stained for senescence-associated β-galactosidase activity as described [10]. Genomic DNA was harvested from early- and late-passage cultures and mean telomere restriction fragment (TRF) length was determined as described [14]. Multicycle FACS analysis was performed as described [40].

Supplementary material

A complete description of all experimental procedures used in this study, including cell culture, analyses, microarray fabrication and performance data, listing of genes and expressed sequence tags (ESTs), and serum induction profiles is available at http://current-biology.com/supmat/supmatin.htm.

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References

1. von Zglinicki T, Saretzki G, Docke W, Lotze C: Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence? *Exp Cell Res* 1995, 220:186-193.
2. Chen Q, Ames BN: Senescence-like growth arrest induced by hydrogen peroxide in human diploid fibroblast F65 cells. *Proc Natl Acad Sci USA* 1994, 91:4130-4134.
3. Di Leonardo A, Linke SP, Clarkin K, Wahl GM: DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. *Genes Dev* 1994, 8:2540-2551.
4. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW: Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 1997, 88:593-602.
5. Lin AW, Barradas M, Stone JC, van Aelst L, Serrano M, Lowe SW: Premature senescence involving p53 and p16 is activated in response to constitutive MEK/MAPK mitogenic signaling. *Genes Dev* 1998, 12:3008-3019.
6. Zhu J, Woods D, McMahon M, Bishop JM: Senescence of human fibroblasts induced by oncogenic Raf. *Genes Dev* 1998, 12:2997-3007.
7. Kato D, Miyazawa K, Ruas M, Starborg M, Wada I, Oka T, *et al.*: Features of replicative senescence induced by direct addition of antennapedia-p16INK4A fusion protein to human diploid fibroblasts. *FEBS Lett* 1998, 427:203-208.
8. Bertram MJ, Berube NG, Hang-Swanson X, Ran Q, Leung JK, Bryce S, *et al.*: Identification of a gene that reverses the immortal phenotype of a subset of cells and is a member of a novel family of transcription factor-like genes. *Mol Cell Biol* 1999, 19:1479-1485.
9. Sherwood SW, Rush D, Ellsworth JL, Schimke RT: Defining cellular senescence in IMR-90 cells: a flow cytometric analysis. *Proc Natl Acad Sci USA* 1988, 85:9086-9090.
10. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, *et al.*: A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci USA* 1995, 92:9363-9367.
11. Cristofalo VJ, Volker C, Francis MK, Tresini M: Age-dependent modifications of gene expression in human fibroblasts. *Crit Rev Eukaryot Gene Expr* 1998, 8:43-80.
12. Linskens MH, Feng J, Andrews WH, Enlow BE, Saati SM, Tonkin LA, *et al.*: Cataloging altered gene expression in young and senescent cells using enhanced differential display. *Nucleic Acids Res* 1995, 23:3244-3251.
13. Harley CB, Futcher AB, Greider CW: Telomeres shorten during ageing of human fibroblasts. *Nature* 1990, 345:458-460.
14. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, *et al.*: Extension of life-span by introduction of telomerase into normal human cells. *Science* 1998, 279:349-352.
15. Vaziri H, Benchimol S: Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. *Curr Biol* 1998, 8:279-282.
16. Prigge ST, Kolhekar AS, Eipper BA, Mains RE, Amzel LM: Amidation of bioactive peptides: the structure of peptidylglycine alpha-hydroxylating monooxygenase. *Science* 1997, 278:1300-1305.
17. Phillips DJ, de Kretser DM: Follistatin: a multifunctional regulatory protein. *Front Neuroendocrinol* 1998, 19:287-322.
18. Tanaka T, Urade Y, Kimura H, Eguchi N, Nishikawa A, Hayaishi O: Lipocalin-type prostaglandin D synthase (beta-trace) is a newly recognized type of retinoid transporter. *Biol Chem* 1997, 272:15789-15795.
19. Dulic V, Drullinger LF, Lees E, Reed SI, Stein GH: Altered regulation of G1 cyclins in senescent human diploid fibroblasts: accumulation of inactive cyclin E-Cdk2 and cyclin D1-Cdk2 complexes. *Proc Natl Acad Sci USA* 1993, 90:11034-11038.
20. Meyyappan M, Wong H, Hull C, Riabowol KT: Increased expression of cyclin D2 during multiple states of growth arrest in primary and established cells. *Mol Cell Biol* 1998, 18:3163-3172.
21. Hara E, Smith R, Parry D, Tahara H, Stone S, Peters G: Regulation of p16CDKN2 expression and its implications for cell immortalization and senescence. *Mol Cell Biol* 1996, 16:859-867.
22. Alcorta DA, Xiong Y, Phelps D, Hannon G, Beach D, Barrett JC: Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. *Proc Natl Acad Sci USA* 1996, 93:13742-13747.
23. Robles SJ, Adami GR: Agents that cause DNA double strand breaks lead to p16INK4a enrichment and the premature senescence of normal fibroblasts. *Oncogene* 1998, 16:1113-1123.
24. Iyer VR, Eisen MB, Ross DT, Schuler G, Moore T, Lee JCF, *et al.*: The transcriptional program in the response of human fibroblasts to serum. *Science* 1999, 283:83-87.
25. West MD: The cellular and molecular biology of skin aging. *Arch Dermatol* 1994, 130:87-95.

26. Martin P: Wound healing—aiming for perfect skin regeneration. *Science* 1997, 276:75-81.
27. Mukaida N, Harada A, Matsushima K: Interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF/MCP-1), chemokines essentially involved in inflammatory and immune reactions. *Cytokine Growth Factor Rev* 1998, 9:9-23.
28. Kirman I, Vainer B, Nielsen OH: Interleukin-15 and its role in chronic inflammatory diseases. *J Inflamm Res* 1998, 47:285-289.
29. Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, Janeway CA Jr: MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell* 1998, 2:253-258.
30. Washizu J, Nishimura H, Nakamura N, Nimura Y, Yoshikai Y: The NF-kappaB binding site is essential for transcriptional activation of the IL-15 gene. *Immunogenetics* 1998, 48:1-7.
31. Seshadri T, Campisi J: Repression of c-fos transcription and an altered genetic program in senescent human fibroblasts. *Science* 1990, 247:205-209.
32. Pang JH, Chen KY: Global change of gene expression at late G1/S boundary may occur in human IMR-90 diploid fibroblasts during senescence. *J Cell Physiol* 1994, 160:531-538.
33. Cooper CL, Newburger PE: Differential expression of Id genes in multipotent myeloid progenitor cells: Id-1 is induced by early-and late-acting cytokines while Id-2 is selectively induced by cytokines that drive terminal granulocytic differentiation. *J Cell Biochem* 1998, 71:277-285.
34. Fisher GJ, Datta SC, Talwar HS, Wang ZQ, Varani J, Kang S, Voorhees JJ: Molecular basis of sun-induced premature skin ageing and retinoid antagonism. *Nature* 1996, 379:335-339.
35. Fisher GJ, Talwar HS, Lin J, Lin P, McPhillips F, Wang Z, *et al.*: Retinoic acid inhibits induction of c-Jun protein by ultraviolet radiation that occurs subsequent to activation of mitogen-activated protein kinase pathways in human skin in vivo. *Clin Invest* 1998, 101:1432-1440.
36. Hjelmeland LM: Senescence of the retinal pigmented epithelium. *Invest Ophthalmol Vis Sci* 1999, 40:1-2.
37. Van Antwerp DJ, Martin SJ, Verma IM, Green DR: Inhibition of TNF-induced apoptosis by NF-kappa B. *Trends Cell Biol* 1998, 8:107-111.
38. Sonenshein GE: Rel/NF-kappa B transcription factors and the control of apoptosis. *Semin Cancer Biol* 1997, 8:113-119.
39. Wang E: Senescent human fibroblasts resist programmed cell death, and failure to suppress bcl2 is involved. *Cancer Res* 1995, 55:2284-2292.
40. Jiang XR, Jimenez G, Chang E, Frolkis M, Kusler B, Sage M, *et al.*: Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype. *Nat Genet* 1999, 21:111-114.

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