Supplementary material

Material and methods

RNA isolation, microarray hybridization, and data analysis

Cells were lysed with TRIzol reagent (Invitrogen, Carlsbad, CA) and total RNA was isolated according to the manufacturer's instructions. RNA integrity was assessed by the presence of 28S and 18S bands in denaturing agarose gel eletrophoresis and spectrophotometry with A260/A280 ratios > 2.0 after dilution in TE-buffer. Independent isolates of young and senescent MRC5 fibroblasts were reverse-transcribed and fluorescence-labeled with Cy3 or Cy5 followed by hybridization onto 70-mer oligonucleotide microarrays printed by the Advanced Technology Center of the NCI featuring 21,329 probes (Qiagen Human Array-Ready Oligo Set, Version 2.0). A total of six arrays, including biological and technical replicates were hybridized as described (1). Hybridized arrays were scanned with a GenePix 4000A scanner (Axon Instruments, Foster City, CA) with variable voltages to achieve saturated signal intensities for about 1% of the total spot number. Acquired images were processed with GenePix Pro 3.0 software (Axon Instruments) and the resulting raw data ratios were uploaded to the NCI MicroArray Database (madb, http://nciarray.nci.nih.gov/) for normalization, data extraction, and further analysis. Microarrays data have been referenced into GEO under the number GSE15919.

Genes (2902) that were present in less than 50% arrays were excluded from further analysis and those with average ratio >= 2 or <= 0.5 were selected, yielding a filtered senescence gene expression signature of 177 upregulated gene probes and 338 downregulated gene probes. This signature was analyzed for features mapped to a KEGG Pathway and/or BioCarta Pathway, using the Pathway Summary Report tool in madb.

RT-PCR analysis.

Sequences of the primers used are CCT-ATC-ACT-GCT-GGC-CTT-TT (WNT16B F), AGC-CGC-TCC-TCT-TTT-GCA-C (WNT16B R), GAC-ACC-ACT-GGA-GGG-TGA-CT (P21), CAG-GTC-CAC-ATG-GTC-TTC-CT (P21 R), ATG-GAG-GAG-CCG-CAG-TCA-

GAT (P53 F), ACC-TGG-GTC-TTC-AGT-GAA-CCA-TTG (P53 R), CGA-GAT-CCC-TCC-AAA-ATC-AA (GAPDH F), ATC-CAC-AGT-CTT-CTG-GGT-GG (GAPDH R), GGG-TTT-AGA-CCG-TCG-TGA-GA (28S F), CCA-TGG-CAA-CAA-CAC-TAC-AT (28S R), CAC-CCT-CAG-CAT-GTT-CAT-TG (PAI-1 F), GGT-CAT-GTT-GCC-TTT-CCA-GT (PAI-1 R), CTC-TCG-GCA-GAG-AAT-TCC-AC (WIG1 F), CCT-GTA-CCG-CTG-TTC-AGA-CA (WIG1 R), CAT-GCT-CTT-CTG-TGG-CTT-CA (PERP F), AAA-GCC-GTA-GGC-CCA-GTT-AT (PERP R), AGC ATG GAG CCT TCG GCT GAC T (P16 F), CTG TAG GAC CTT CGG TGA CTG AT (P16 R).

1. Staib F, Robles A, Varticovski L, et al. The p53 tumor suppressor network is a key responder to microenvironmental components of chronic inflammatory stress. Cancer Res 2005; 65: 10255-64.

Figure legends

Table S1.

Microarray analysis of genes expression in replicative senescence in MRC5 fibroblasts.

Table S2.

Microarray analysis of genes upregulated in replicative senescence in MRC5 fibroblasts: enriched KEGG pathways.

Table S3.

Microarray analysis of genes downregulated in replicative senescence in MRC5 fibroblasts: enriched KEGG pathways.

Figure S4.

WNT16B regulation in replicative senescence.

Pre-senescent MRC5 fibroblasts (PDL58) were stably infected with retrovirus coding for the human telomerase reverse transcriptase catalytic unit (hTERT).

A - MRC5 hTERT fibroblasts were stained for SA-β-Gal expression (left) and quantified (right). Percentage of positive cells from MRC5 PDL64 is shown in lack. The graphs represent means ± standard deviation (SD).

B - WNT16B and p21 mRNA expression in young (PDL26), senescent (PDL64), or hTERT MRC5 fibroblasts was analyzed by qRT-PCR. 18S was used as control. mRNA expression in MRC5 PDL26 fibroblasts was normalized to 1. The graphs represent means ± SD.

C - Western blot analysis of WNT16, p53, and p21 protein expression using β-actin as a loading control.

Figure S5.

Overexpression of WNT16B in young fibroblasts – analysis of senescence markers.

Young MRC5 fibroblasts (PDL26) were infected with a retrovirus coding for WNT16B (pBabe-WNT16B).

A – One hundred thousand selected cells were tested for SA-β-gal activity and quantified. The graphs represent means ± SD

B – Five thousand cells were seeded into 10cm² plates for 3 weeks and stained with methylene blue.

C – BrdU incorporation into pBabe and pBabe-WNT16B MRC5 fibroblasts.

D – *Left*: WNT16B, p53, and p21 mRNA expression was analyzed by qRT-PCR in pBabe-WNT16B MRC5 fibroblasts. 18S was used for standardization. mRNA expression in MRC5 PDL26 fibroblasts was normalized to 1. The graphs represent means ± SD. *Right*: Analysis of WNT16 and p16INK4a proteins expression by western blot. α-tubulin was used as loading control.