SUPPLEMENTARY METHODS

Real-time PCR. hDMP1α, p53, $p21^{WAF1/CIP1}$ and wig-1 transcripts were measured using the UniPrimer detection system (1), $p14^{ARF}$ and ABL were measured using primers and probes. Primer sequences and real-time RT-PCR are described in supplemental methods. Briefly, the 5' end of the target-specific forward primer contained a tail complementary to the 3' end of the UniPrimer (see nucleotides depicted in lower case in forward primers below; Chemicon, Temecula, CA). This hairpin primer was labeled with a fluorescent energy transfer pair; upon amplification of the target gene, the hairpin unfolded and fluorescence was activated. The primer sequences were as follows: for hDMP1α, forward 5'-actgaacctgaccgtacaAGAAGCTCAAGGAGCTCCGGATA-3' and reverse 5'-CCCTATTGTTGCCCAGTCATTG-3'; for p53, forward 5'-actgaacctgaccgtaca GCGTGAGCGCTTCGAGAT-3' and reverse 5'-CAGCCTGGGCATCCTTGA-3'; for $p21^{WAF1/CIP1}$, 5'-actgaacctgaccgtacaCTGGAGACTCTCAGGGTCGAA-3' and reverse 5'-CGGCGTTTGGAGTGGTAGAA-3'; and for wig-1, 5'-actgaacctgaccgtacaGCCTGCAGCTACTCCAGTTGT-3' and reverse 5'-CACTCGGCCTCCTGGCTTA-3'. The following primer and probe combinations were used to analyze ABL and $p14^{ARF}$: for ABL, forward 5'-TGTGGCCAGTGGAGATAACACT-3', reverse 5'-CCATTCCCCATTGTGATTATAGC-3', and probe 6FAM-5'-TAAGCATAACTAAAGGTGAAAAGCTCCGGGTCTTA-3'-TAMRA; and for $p14^{ARF}$, forward 5'-AGCAGCCGCTTCCTAGAAGAC-3', reverse 5'-

CACGGGTCGGGTGAGAGT-3', and probe 6FAM-5'-CGAGTGGCGGAGCTGCTGCT-3'-TAMRA (2). Samples were analyzed using standard conditions on a 7900HT sequence detection system (Applied Biosystems, Foster City, CA). Specific gene expression was normalized to ABL expression to correct for amount of total RNA input. Expression was quantified according to the comparative threshold method using the formula $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta Ct(sample) - \Delta Ct(calibrator)$, and ΔCt is the Ct of the target gene subtracted from the Ct of the house keeping gene ABL (3). Target gene expression of HeLa cells transduced with the VP activation domain was used as calibrator and results are given as fold expression relative to this level of expression.

Total RNA extraction and RNA quality assessment for microarray profiling

Cell lysate was homogenized by passing over a QIAshredder spin column (Qiagen, Valencia, CA) and total RNA was isolated from the cell pellet using the RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. RNA quantity and quality was analyzed using the RNA 6000 Nano Assay (Agilent Technologies, Palo Alto, CA) according to the protocol recommended by the manufacturer. The computational analyses were performed with the Agilent 2100 Bioanalyzer software (Agilent Technologies, Palo Alto, CA).

Microarray processing and data analysis

RNA samples were processed for hybridization on Affymetrix HG-U133A microarrays following procedures recommended by the manufacturer. Microarray data were retrieved as MAS5 flat files and imported into GeneSpring 6.1 (Silicon Genetics, Palo Alto, CA) or Excel (Microsoft).

Affymetrix MAS5 data were normalized to a constant value of 1 in GeneSpring, effectively keeping the global normalization to a target intensity of 150 using the MAS5 algorithm. For clustering analyses, the experimental interpretation was set to “log of ratio” and Pearson correlation was used as a similarity measure for both dimensions (gene tree and experiment tree). To derive differentially expressed genes, a parametric test, not assuming equal variances (Welch t-test), was used in the “log of ratio” mode. No multiple testing correction was applied. Fold change filtering was performed either in GeneSpring with the experiment interpretation set to “ratio” or in Excel. Genes were only considered as differentially expressed when the majority of measurements corresponded to “present” or “marginal” calls in at least one group of experiments. Specific cut-off values for the various filtering steps are given in the results section.

Preparation of lentiviral transducing particles and transduction

Cloning of the Hygromycin B resistant or the combined p53 siRNA/Hygromycin B resistant lentiviral transfer vectors, pCR-XL-CS-hygro and pCR-XL-CS-sip53hygro, was described (4). VSV-G-pseudotyped lentiviral

supernatant was generated by transiently transfecting the transfer vectors and the following third generation packaging system: VSV-G-expressing plasmid pMD.G, *gag* and pol expressing pMDLg/p.RRE and rev expressing pRSV-Rev plasmids as described (5). HeLa cells were transduced over night in the presence of 8 µg/ml polybrene using lentivirus at an MOI of 10, thereafter washed and maintained as described above. The transduction was repeated once and two days later polyclonal pools were selected for 10 days in 300 µg/ml Hygromycin B (Roche, Indianapolis, IN). Selected cells were maintained in complete medium with 100 µg/ml Hygromycin B.

References

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