SUPPLEMENTAL INFORMATION

E2F1-CIP2A feedback loop prevents senescence induction in breast cancer cells with inactive p53

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SUPPLEMENTARY MATERIALS AND METHODS

RNAi and adenoviral transductions

MCF-7 and MDA-MB-231 cells were transfected at 30% confluency with 250pmol of siRNA by using Oligofectamine (Invitrogen) according to manufacturer's instructions. siRNA sequences are presented in Table S2. Stable control (shNTC, non targeting control) and E2F1 shRNA expressing MCF-7 cells were generated by lentiviral transduction according to standard protocol and MCF-7 cells were selected with puromycin (2,5µg/ml) for 7 days. MCF-7 and MDA-MB-231 cells were transduced with adenoviruses at 30-40% confluency with medium supplemented with 1% FCS. 24h after transduction medium was changed to 10% FCS. CIP2A together with RFP was cloned into human adenoviral type 5 vector under the control of CMV promoter (Vector Biolabs). p21 adeno expressing p21 and a control virus RFP adeno expressing RFP under CMV promoter control in adenovirus type 5 were purchased from Vector Biolabs. p53 and control lacZ adeno viruses have been described previously (Ala-aho et al., 2002).

RT-PCR analysis

For RNA analysis, total RNA were isolated from cultured cells and from mouse tissue samples by using NucleoSpin II kit including DNAse treatment (Machanerey-Nagel). RNA isolation was performed according to manufacturer's instructions. cDNA was produced from RNA using M-MuLV RNase H minus reverse transcriptase (Finnzymes). RT-PCR analysis was performed using ABI 7900HT Fast Sequence Detection and Taqman Universal Master Mix II (Applied Biosystems). Expression of each studied transcript were normalized to β-actin reference gene. To analyze RNA expression of different senescence related genes, RT^2 Profiler^{TM} PCR Array Human/Mouse Cellular Senescence, RT^2 SYBR Green/ROX PCR Master mix (SA Biosciences) and ABI 7900HT Fast Sequence Detection was used (Applied Biosystems). The data was normalized to geometric mean of five reference genes B2M, HPRT1, RPL13A, GAPDH and ACTB for human and Gusb, Hprt, Hsp90ab1, Gapdh and Actb for mouse samples. RT-PCR primers to detect B55α and B56β are previously published (Sablina et al. 2010). All RT-PCR expression analysis were performed at The Finnish Microarray and Sequencing Centre (FMSC), Turku, Finland.

ChIP and identification of putative p53 and E2F1 binding sites at CIP2A promoter

Putative p53 responsive elements at CIP2A promoter were defined using Genomatix software and a promoter alignment analysis tool ConTra (http://bioit.dmbr.ugent.be/ConTra/index.php). Chromatin immunoprecipitation (ChIP) was performed using HCT116 cells treated with 0.2μg/ml of

doxorubicin. ChIP assays were performed using protocol adapted from Ohkubo et al. 2006. For ChIP mouse monoclonal DO-1 p53 antibody (Santa Cruz Biotechnology) was used. Primers used to analyze both input and immunoprecipitated DNA by RT-PCR are listed in Table S1. DNA levels and errors on DNA levels were calculated using qBasePLUS 1.0 analysis software (http://www.biogazelle.com) that uses a delta–quantification cycle (delta–Cq) model normalisation to multiple reference sequences. Genomic regions of the promoters of β-actin, HMBS, and TBP, were used for normalisation. ChIP was performed twice with similar results. A putative E2F1 binding site at CIP2A promoter was defined by using Genomatix software. ChIP was performed by transfecting Saos-2 cells either with empty pCMV plasmid or E2F1 expressing pCMV plasmid. Cells were harvested and ChIP was performed according to manufacturers instructions with the Chromatin Immunoprecipitation Assay Kit (Millipore). The antibodies used for ChIP were mouse monoclonal E1a (BD Pharmingen) as a non-specific control and rabbit polyclonal C-20 E2F1 (Santa Cruz Biotechnology). Primers used to analyze both input and immunoprecipitated DNA by RT-PCR are listed in Table S2.

Luciferase reporter assays

CIP2A promoter luciferase construct (Khanna et al., 2011) or EGFR promoter luciferase construct (Johnson et al., 2000) was co-transfected with a control plasmid Ubi-Renilla luciferase construct to MCF-7 cells on 96-well plate by using Fugene transfection reagent (Roche) according to manufacturer's protocol. 24h after transfection cells were treated with Nutlin-3 for 24h. In a case of CIP2A rescue, MCF-7 cells were transduced on 96-well plate either

with control or CIP2A expressing adenovirus 24h before transfection of MYC responsive luciferase reporter (Ayer et al., 1996). Firefly and renilla luciferase activities were measured with Dual-Glo Luciferase Assay System (Promega) and renilla luciferase values were used for data normalization.

Ingenuity analysis

Transcription factor analysis of CIP2A regulated gene expression signature (Niemela et al., 2012) was conducted by Ingenuity pathway analysis (Ingenuity Systems) according to their standard procedures. The z-score predicts activation state of the transcription factor. A z-score more than +2 (transcription factor activated) or less than -2 (transcription factor inhibited) was considered significant.

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