Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin resistant human testicular carcinoma cells via the Fas/FasL pathway

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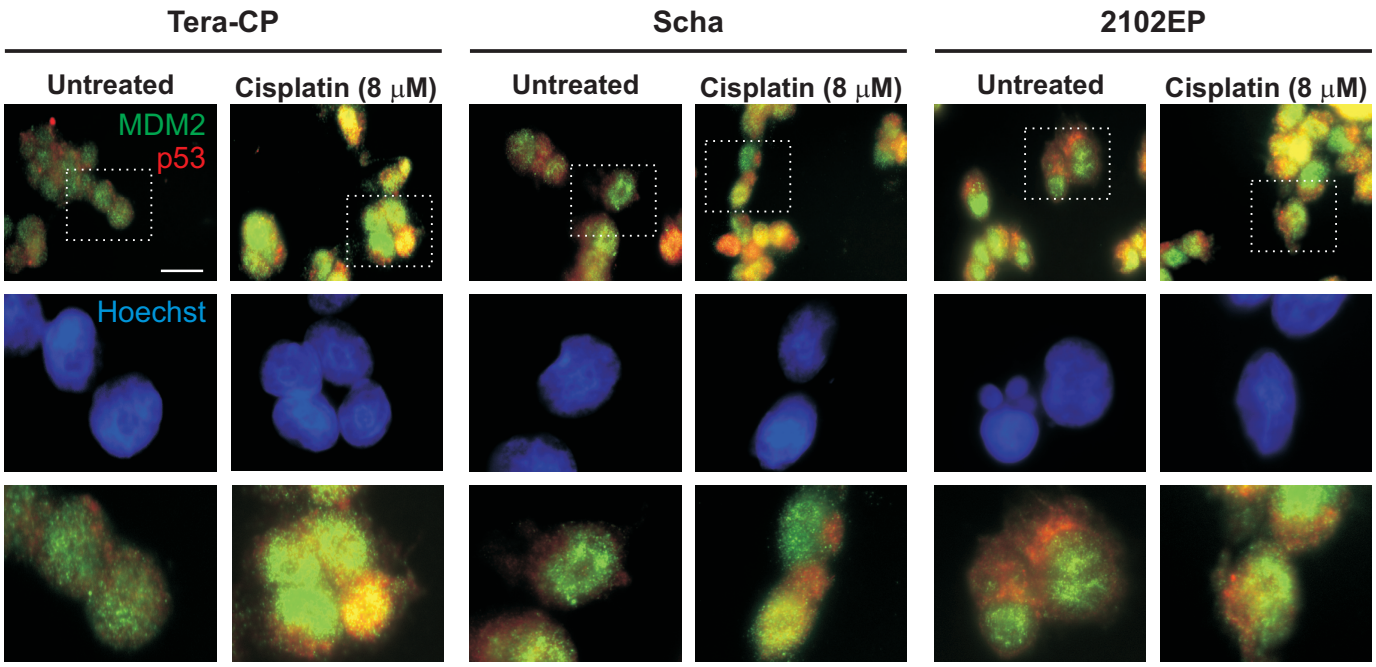
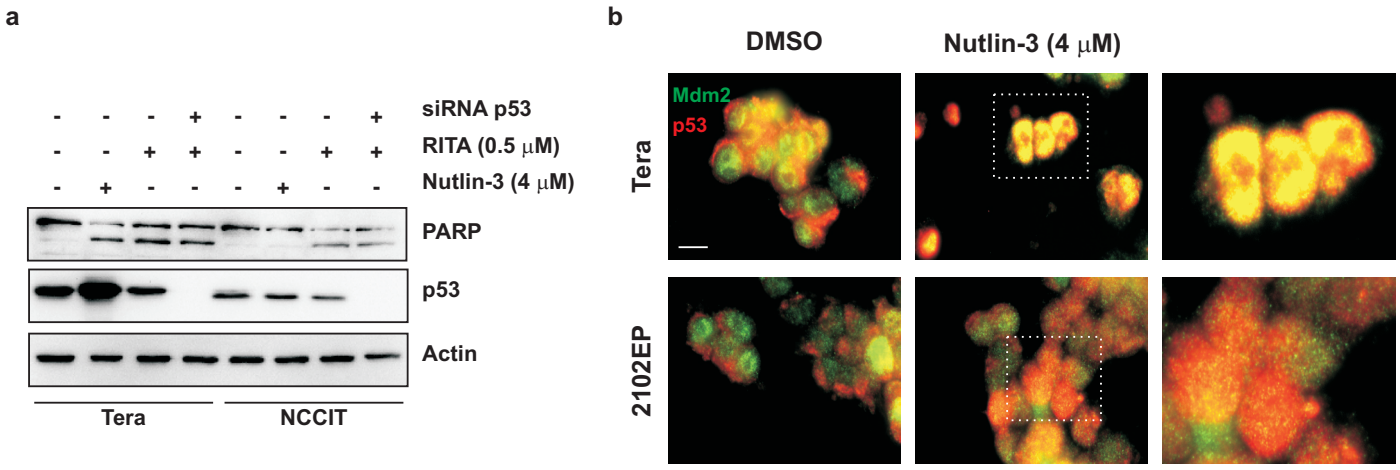
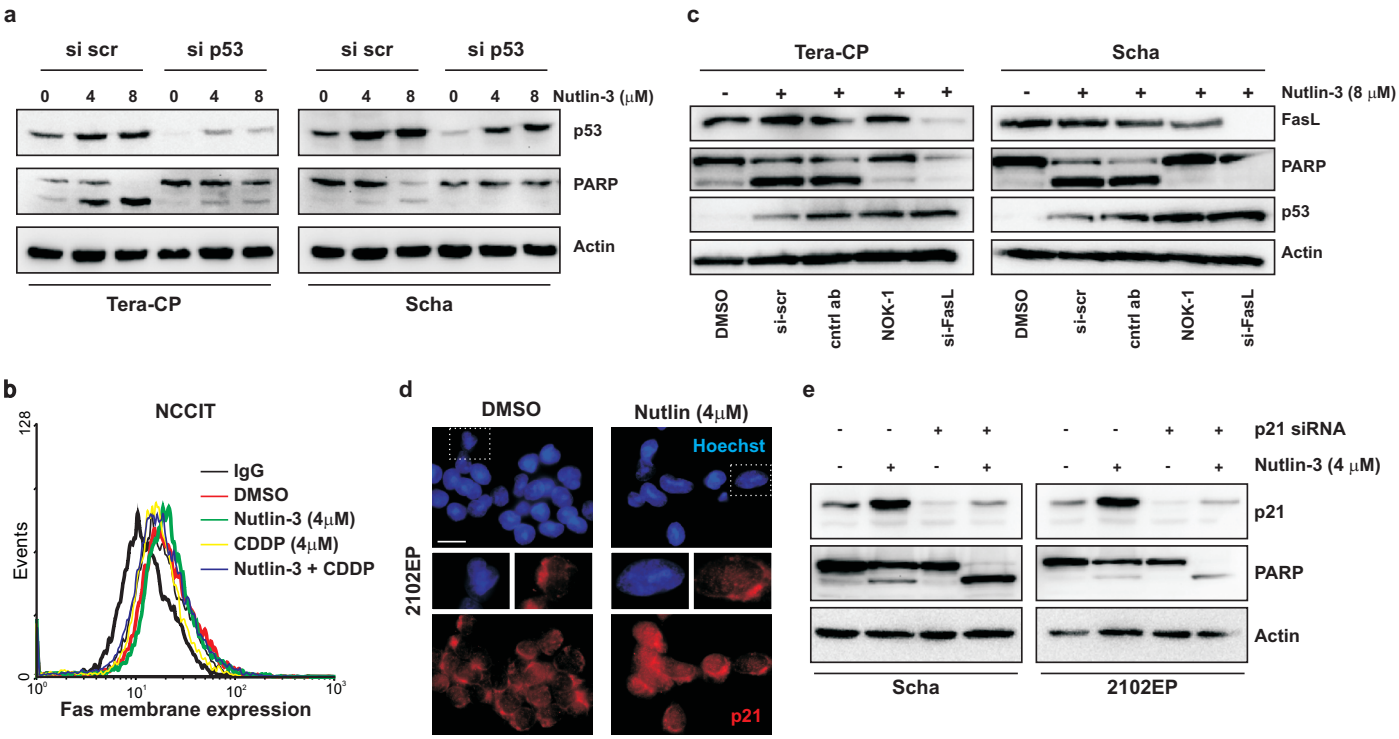


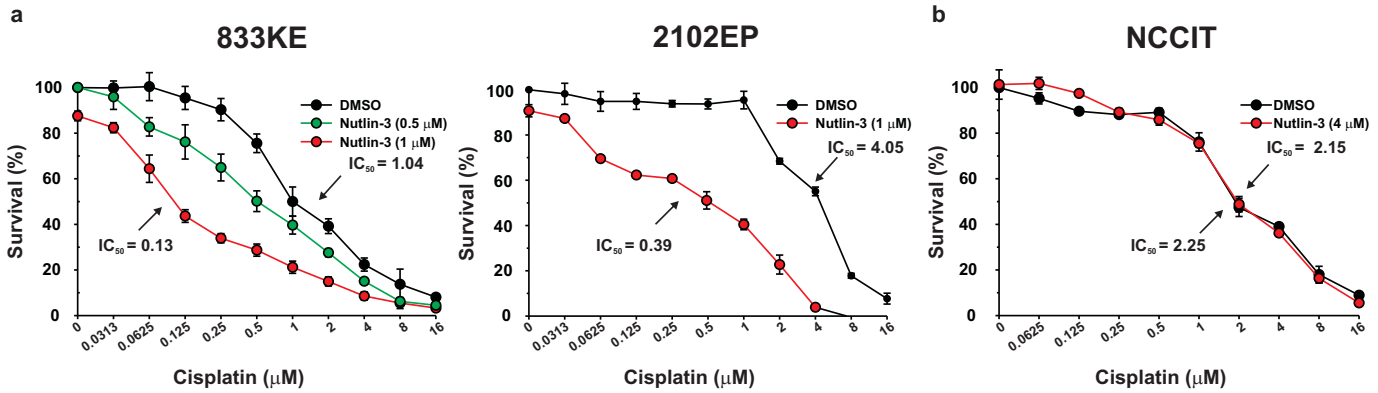
Supplemental Figure 1. Immunofluorescence showing cytoplasmic localised p53, while MDM2 maintains nuclear localised after cisplatin treatment (8 μM) in the cisplatin-resistant TC cell lines Tera-CP, Scha and 2102EP; a representative example of three independent experiments is shown. Selected area of the original image, as indicated, x2.5 digitally magnified. Scale bar: 30μm.



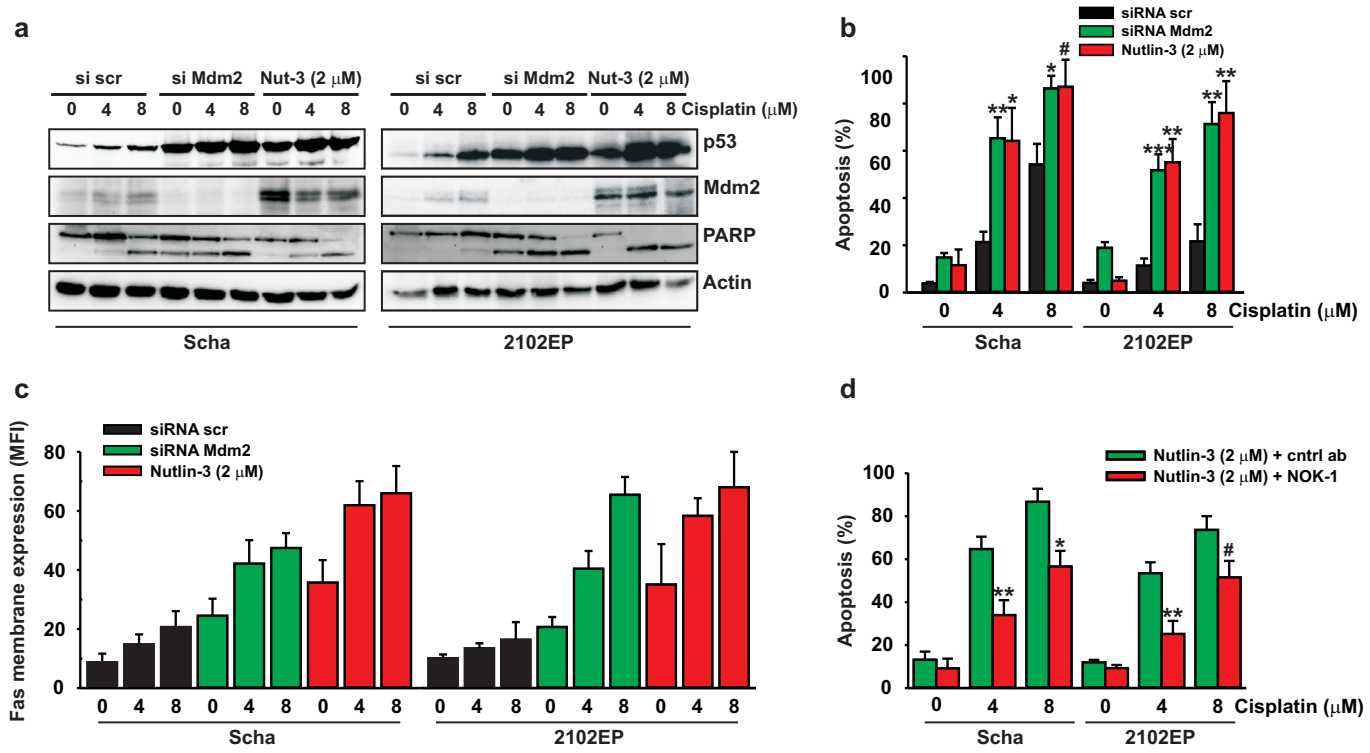
Supplemental Figure 2. (a) No induction of p53 is observed after treatment with RITA. Suppression of p53 with a specific siRNA does not interfere with the apoptosis induction by RITA as visualised by PARP cleavage; a representative example of three independent experiments is shown. (b) Immunofluorescence shows that p53 becomes more prominently nuclear localised after Nutlin-3 treatment in the cisplatin-sensitive TC cellline Tera and the intrinsically cisplatin-resistant cell line 2102EP compared to control; a representative example of three independent experiments is shown. Selected area of the original image, as indicated, x2.5 digitally magnified. Scalebar: 30μm.



Supplemental Figure 3. (a) Successful downregulation of p53 using siRNA targeting p53, and decreased cleavage of PARP in p53-suppressed Nutlin-3 treated TC cells compared to control; a representative example of three independent experiments is shown. (b) Unchanged Fas membrane expression of the mutant p53 cell line NCCIT following Nutlin-3 and/or cisplatin treatment; a representative example of three independent experiments is shown. (c) Reduced PARP cleavage in Tera-CP and Scha after successful downregulation of FasL or blocking of FasL, with Nok-1; a representative example of three independent experiments is shown. (d) Immunofluorescence shows that p21 is mainly cytoplasmic localized after DMSO (control) and Nutlin-3 treatment in the cisplatin-resistant TC cell line 2102EP; a representative example of three independent experiments is shown. Selected area, as indicated, of the original image x4 digitally magnified. Scale bar: 30 μm. (e) Downregulation of p21 increases the apoptotic response of the intrinsically cisplatin-resistant cells Scha and 2102EP after Nutlin-3 treatment; a representative example of three independent experiments is shown.



Supplemental Figure 4. (a-b) Survival of TC cells after 96h of continuous Nutlin-3 treatment as indicated, in combination with increasing cisplatin concentration. IC_{50} values are depicted for cisplatin as well as the combination; values are the mean ± SD.



Supplemental Figure 5. (a) Increased levels of p53 and increased PARP cleavage after targeting the MDM2/p53 axis; a representative example of three independent experiments is shown. (b) Increased apoptosis after targeting the MDM2/p53 axis with either siRNA against MDM2 or Nutlin-3 (Nut-3) in combination with cisplatin. Values are the mean ± SD of three experiments; #$p < 0.05$; *$p < 0.01$; **$p < 0.005$. (c) Following the indicated treatment TC cells were harvested and Fas membrane expression was determined by flow cytometry. Values were depicted as mean fluorescence intensity (MFI). Values are the mean ± SD of three experiments. (d) Decreased apoptotic response after blocking of FasL, with Nok-1, in TC cells treated with the combination of cisplatin and Nutlin-3. Values are the mean ± SD of three experiments; #$p < 0.05$; *$p < 0.01$; **$p < 0.005$.

Supplemental Materials and Methods

Antibodies

The following antibodies were used: mouse anti p53 (DO-1, Santa Cruz), mouse anti-Mdm2 (SMP14, Oncogene Research Products, Darmstadt, Germany), mouse anti β-Actin (MP Biomedicals, Eindhoven, the Netherlands), mouse anti p21 (F5, Santa Cruz), rabbit anti-Parp (Roche Diagnostics, Almere, the Netherlands), caspase 8 (1C12, Cell Signalling, MA, USA), and anti-FasL (C20, Santa Cruz).

RNA interference sequences

Sequence for p53 I small interfering RNA (siRNA) molecules was 5'-GCA UGA ACC GGA GGC CA UdTdT-3' (sense) and 5'-AUG GGC CUC CGG UUC AUG CdTdT-3' (anti-sense), sequence for p53 II siRNA was 5'-CUU CGA CUU UGU CAC CGA GdTdT-3' (sense) and 5'-CUU ACG CUG AGU ACU UCG AdTdT-3' (anti-sense), sequence for P21 I siRNA was 5'-GAC CAU GUG GAC CUG UCA CTdT-3' (sense) and 5'- GUG ACA GGU CCA CAU GGU CdTdT-3' (anti-sense), sequence for P21 II siRNA was 5'-CUU CGA CUU UGU CAC CGA GTdT-3' (sense) and 5'-CUC GGU GAC AAA GUC GAA GTdT-3' (antisense), sequence for FasL siRNA was 5'-CTG GGC TGT ACT TTG TAT AdTdT-3' (sense) and 5'-TAT ACA AAG TAC AGC CCA GdTdT-3' (anti-sense), sequence for MDM2 siRNA was 5'-GTG AAT CTA CAG GGA CGC CAT CdTdT-3' (sense) and 5'-GAT GGC GTC CCT GTA GAT TCA CdTdT-3' (anti-sense).