

Ambrosio et al.

Carbosilane dendrimers loaded with siRNA targeting Nrf2 as a tool to overcome cisplatin chemoresistance in bladder cancer cells.

SUPPLEMENTARY MATERIALS

Fig 1S. CDDP-resistance and Nrf2 expression in bladder cancer cells

Both CDDP-resistance and Nrf2 expression was checked in T24 and 253J B-V C-r cells, to confirm our previously reported data (4). As shown in **Fig 1S**, panel A, 2.5 $\mu\text{g/ml}$ CDDP did not affect cell viability (by MTT test) in both T24 and 253J B-V C-r at any time tested, while the inhibition in the sensitive 253J B-V parental clone was statistical significant since 24 h. Higher basal expression of Nrf2 protein in CDDP-resistant cells, with the respect to the sensitive 253J B-V parental clone, was also confirmed (**Fig. 1S**, panel B).

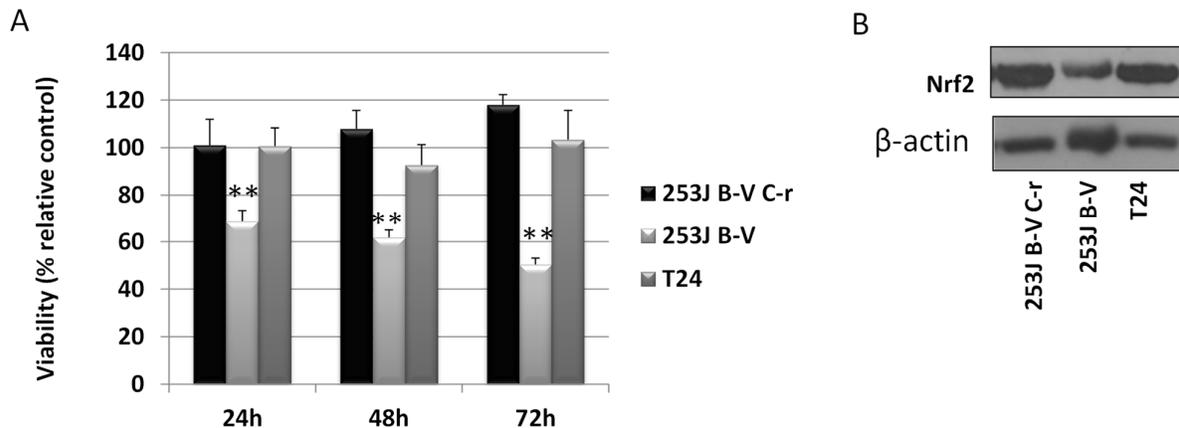


Fig. 1S Panel A: MTT assay in T24, 253J B-V C-r, 253J B-V cells treated with 2.5 $\mu\text{g/ml}$ CDDP. Viability was checked at the indicated times. Results are expressed as percent of the relative control values and are the mean \pm standard deviation of three separate experiments performed in triplicate. ** = $p < 0.01$ versus relative control value. Panel B: Western blot analysis of Nrf2 in T24, 253J B-V C-r, and 253 J B-V. Equal protein loading was confirmed by exposure of the membranes to the anti-GAPDH antibody.

Fig. 2S. Nrf2 and Gsta4 inhibition with a lower amount of siNrf2-GCD

T24 and 253J B-V C-r cells were treated with siNrf2-GCD alone (final concentrations 4 µg/ml GCD, 0.04 µM siNrf2) or with naked siNrf2 4 µg/ml transfected the HiPerFect® reagent. Results showed that a lower amount of siNrf2-GCD was able to inhibit Nrf2. Transfection with the HiPerFectR reagent, used as a positive control, showed similar inhibitions.

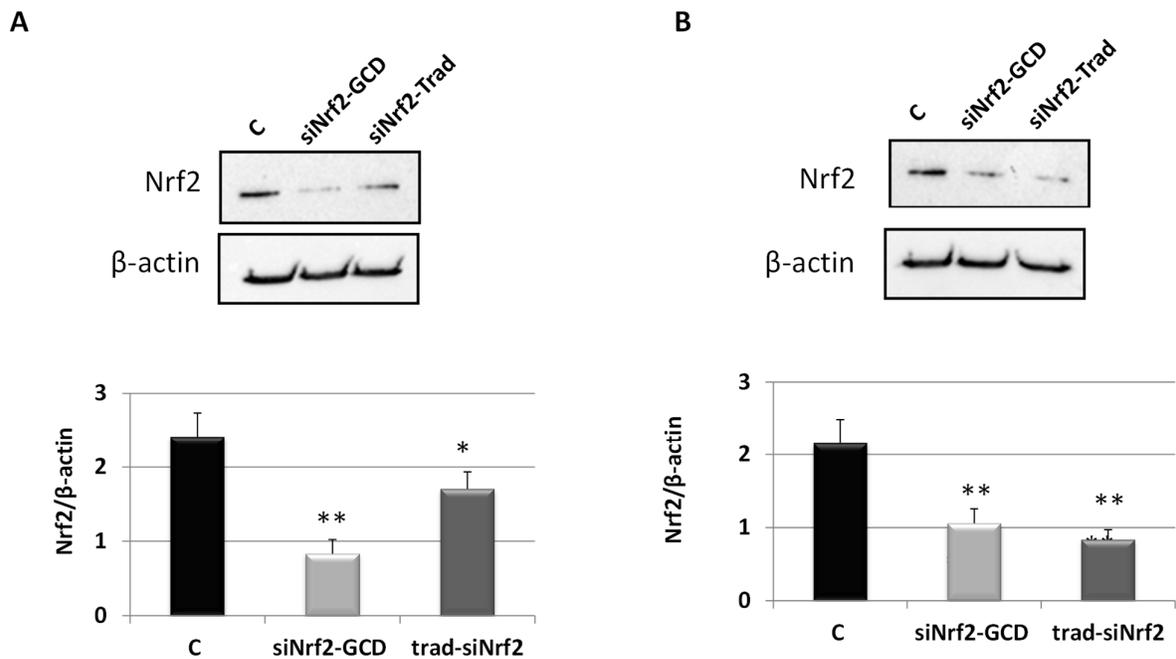


Fig. 2S Western blot analysis of Nrf2 in T24 (A) and 253 J B-V C-r CDDP-resistant cells (B), untreated (control, C), treated with siNrf2-GCD (0.04 µM siNrf2 in 4 µg/ml GCD), transfected with the same amount of siNrf2 (0.04 µM) in HiPerFect® reagent with the traditional method (siNrf2-Trad). Cells were collected after 24 h from the treatments. Below, relative densitometric scanning of Nrf2 expression normalized using the β-actin signal. Data are the mean ± SD from three independent experiments. **p<0.01, *p<0.05 vs. control.