4-2020

TIP60 regulation of ΔNp63α is Associated with Cisplatin Resistance

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TIP60 regulation of ΔNp63α is associated with Cisplatin resistance

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Abstract

ΔNp63α, a member of the p53 transcription factor family, is overexpressed in non-melanoma skin cancer and regulates cell survival, migration and invasion. TIP60 is histone acetyltransferase (HAT) which mediates cellular processes such as transcription and the DNA damage response (DDR). Since ΔNp63α is known to transcriptionally regulate several DDR genes and promote cisplatin resistance, its stabilization by TIP60 may contribute to the failure of platinum-based drugs in squamous cell carcinoma (SCC). We hypothesize that TIP60 regulates the transcriptional activity of ΔNp63α thereby modulating chemoresistance. In this study, we showed that overexpression of TIP60 in both H1299 and A431 cells led to increase in the levels of ΔNp63α, while TIP60 silencing in A431 cell lines led to a decrease in endogenous ΔNp63α transcript and protein levels, thus confirming that TIP60 positively regulates ΔNp63α in these cell lines. Increased levels of ΔNp63α and TIP60 were observed in a cisplatin resistant A431 SCC line. Further, stable expression of TIP60 or ΔNp63α individually promoted resistance to cisplatin, whereas loss of ΔNp63α and TIP60 sensitized cells to cisplatin. Higher acetylation of ΔNp63α and TIP60 were seen in cisplatin resistant cells. Taken together, our data suggest that TIP60-mediated regulation of ΔNp63α increases cisplatin resistance and has potential implications for cancer treatment and drug design. Additionally, since ΔNp63α confers cisplatin resistance through regulation of genes involved in DNA damage repair, our findings provide critical insight into the mechanism by which genes involved in cisplatin resistance are regulated and may lead to strategies for treating resistant tumors with increased efficacy.

p63 Isoforms

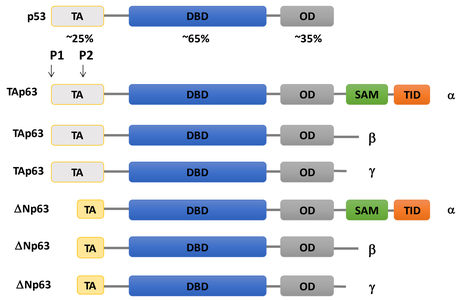


Figure 1: p63 isoforms. Schematic of the p63 gene comprising of the two promoter sites, 3' splicing segments, and the resulting six main p63 isoforms (TAp63α, TAp63β, TAp63γ, ΔNp63α, ΔNp63β and ΔNp63γ). The domains are as follows: transactivation domain (TA), DNA-binding domain (DBD), oligomerization domain (OD), sterile alpha motif (SAM), and transactivation inhibitory domain (TI).

TIP60- a histone acetyltransferase

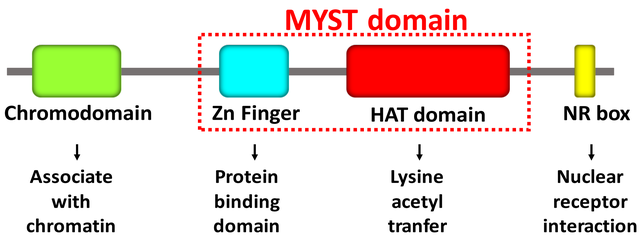


Figure 2: TIP60. Schematic of the TIP60 protein comprising of the domains as follows: Chromodomain, Zinc finger motif (Zn Finger), Histone acetyltransferase domain (HAT domain) and Nuclear receptor box (NR box). The MYST domain, comprised of an acetyl-coenzyme A binding domain and a zinc finger motif, is responsible for catalytic activity.

Hypothesis: TIP60 regulates ΔNp63α to confer cisplatin resistance

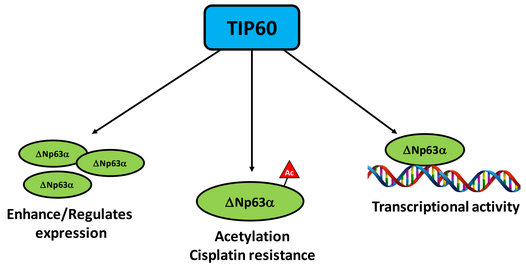


Figure 3: Potential mechanisms by which TIP60 regulates ΔNp63α.

TIP60 silencing reduces ΔNp63α transcript and protein levels

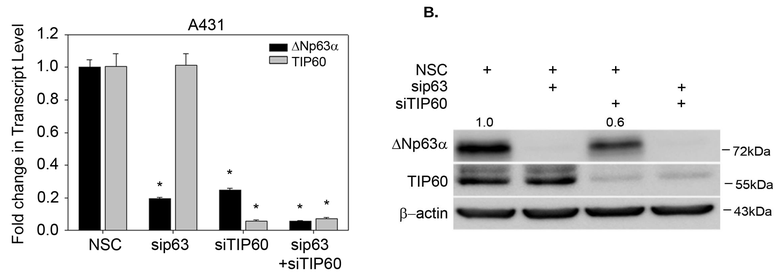


Figure 4: TIP60 silencing reduces ΔNp63α transcript and protein levels. A431 cells were transfected with non-silencing control (NSC) siRNA or siRNA against p63 (sip63) and/or TIP60 (siTIP60) and harvested at 48 hours post-transfection. (A) RNA levels were measured by TaqMan based qRT-PCR with assays on demand (AOD's) specific to ΔNp63α, TIP60 and normalized to GAPDH. Fold-changes are calculated relative to NSC. Error bars represent ±1 standard deviation from the mean. *P<0.05 compared to respective NSC control. (B) Protein levels were measured by immunoblot analysis performed with antibodies specific for p63, TIP60 or β-actin. β-actin was included as a loading control for equivalent protein in each lane.

Cisplatin resistant cells exhibit elevated levels of TIP60 and ΔNp63α

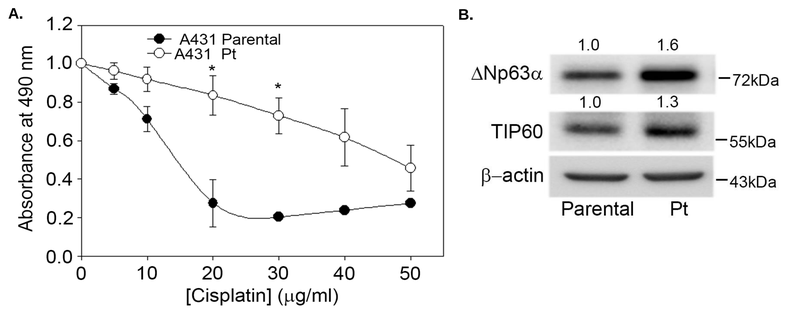


Figure 4: Cisplatin resistant cells exhibit elevated levels of TIP60 and ΔNp63α. (A) A431 Parental (control) and Pt (cisplatin-resistant) cells were subjected to a 2 hr cisplatin pulse using increasing concentrations of cisplatin. At 48 h post-treatment, cell viability was measured by MTS assay. The y-axis indicates fold change compared to vehicle treated cells. Error bars represent ±1 standard deviation from the mean value of two representative experiments. *P<0.05 compared to parental A431 at the same cisplatin dose (B) Immunoblot analysis performed on A431 parental and Pt cells using antibodies specific for ΔNp63α, TIP60 or β-actin. Immunoblot with β-actin was performed to confirm equivalent protein loading. Densitometry quantitation of protein levels relative to Parental cell vehicle controls is indicated.

TIP60 protects ΔNp63α upon Cisplatin treatment

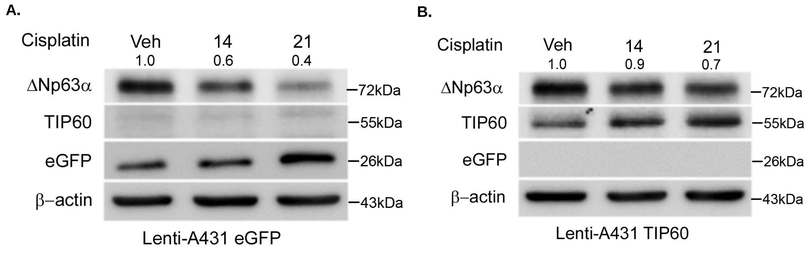


Figure 5:Overexpression of TIP60 protects ΔNp63α levels upon Cisplatin treatment. (A,B) Lenti-A431 e-GFP and Lenti-A431-TIP60 stable cells were treated with either DMSO (vehicle) or at two cisplatin dosage in a 2-hours pulse and harvested 24 hours later. Immunoblot analysis was performed using antibodies specific to ΔNp63α, TIP60, eGFP or β-actin. Immunoblot with β-actin was performed to confirm equivalent protein loading.

Silencing of TIP60 and/or ΔNp63α sensitizes cells to cisplatin

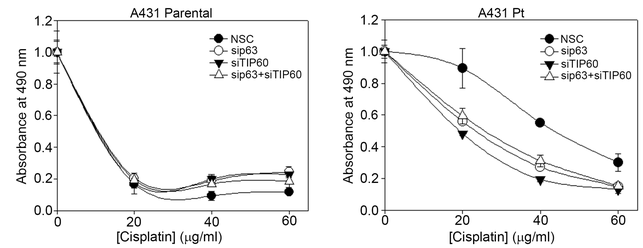


Figure 6: Silencing of TIP60 and/or ΔNp63α sensitizes Pt cells to cisplatin. A431 Parental (control) and cisplatin-resistant (Pt) cells were transfected with non-silencing control (NSC) siRNA or siRNA against p63 (sip63) or TIP60 (siTIP60) followed by treatment with a 2-hour pulse of vehicle at the indicated cisplatin doses. Cell viability was measured by MTS assay at 48 hours. The y-axis indicates the fold change compared to vehicle treated cells. Error bars represent ±1 standard deviation from the mean.

ΔNp63α/TIP60 expression increase resistance to cisplatin

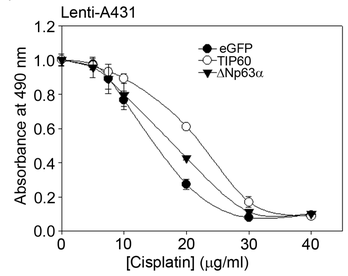
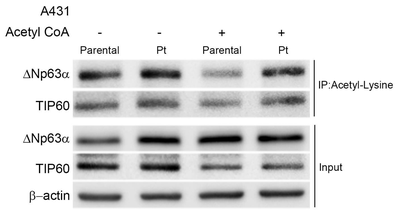


Figure 7: Stable expression of either ΔNp63α or TIP60 increases resistance to cisplatin. Lenti-A431-eGFP (control), ΔNp63α and TIP60 were subjected to a 2-hour cisplatin pulse treatment at the indicated doses. Cell viability was measured by MTS assay at 24 hours. The y-axis indicates fold change compared to the vehicle treated cells. Error bars represent ±1 standard deviation from the mean of two representative experiments.

Acetylation of ΔNp63α is elevated in cisplatin resistant cells

Figure 8: Acetylation of ΔNp63α is elevated in cisplatin resistant Pt cells. A431 Parental (control) and cisplatin-resistant (Pt) cells were treated with 30mM sodium butyrate and 250μM of Acetyl-CoA for 6 hours and harvested 6 hours post treatment. Whole cell lysates were immunoprecipitated (IP) with an acetylated-lysine antibody followed by immunoblot analysis using antibodies specific for ΔNp63α, TIP60 or β-actin.



Summary

- **Cisplatin resistant cells exhibit elevated levels of TIP60 and ΔNp63α**
- **TIP60 protects ΔNp63α upon Cisplatin treatment**
- **Silencing of TIP60 and/or ΔNp63α sensitizes cells to cisplatin**
- **ΔNp63α/TIP60 expression increase resistance to cisplatin**
- **Acetylation of ΔNp63α is elevated in cisplatin resistant cells**

Future Directions

- **Confirm acetylation of ΔNp63α is dependent on Tip60 in cisplatin resistant cells**
- **Validate the finding from NGS study**
- **Study the effects of TIP60 on ΔNp63α transcriptional activity**
- **Identify the genes differentially regulated by ΔNp63α in DNA damage response**

Acknowledgments

The work presented from this project was supported by grant R01 CA154715-02 (MK) from the National Institutes of Health