TAp73 CONTRIBUTES TO THE OXIDATIVE STRESS RESPONSE BY REGULATING PROTEIN SYNTHESIS

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TAp73 regulates mRNA translation

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ABSTRACT

TAp73 is a transcription factor that plays key roles in brain development, ageing and cancer. At the cellular level, TAp73 is a critical homeostasis-maintaining factor, particularly following oxidative stress. Although major studies focused on TAp73 transcriptional activities have indicated a contribution of TAp73 to cellular metabolism, the mechanisms underlying its role in redox homeostasis have not been completely elucidated. Here we show that TAp73 contributes to the oxidative stress response by participating in the control of protein synthesis. Regulation of mRNA translation occupies a central position in cellular homeostasis during the stress response, often by reducing global rates of protein synthesis and promoting translation of specific mRNAs. TAp73 depletion results in aberrant ribosomal RNA (rRNA) processing and impaired protein synthesis. In particular, polysomal profiles show that TAp73 promotes the integration of mRNAs that encode rRNA-processing factors in polysomes, supporting their translation. Concurrently, TAp73 depletion causes increased sensitivity to oxidative stress that correlates with reduced ATP levels, hyperactivation of AMPK and translational defects. TAp73 is important for maintaining active translation of mitochondrial transcripts in response to oxidative stress, thus promoting mitochondrial activity. Our results are the first to indicate that TAp73 contributes to redox homeostasis by affecting the translational machinery, facilitating the translation of specific mitochondrial transcripts. This study identifies a mechanism by which TAp73 contributes to the oxidative stress response and describes a completely unexpected role for TAp73 in regulating protein synthesis.

Significance Statement

Oxidative stress is a critical contributor to ageing-associated diseases, including neurodegeneration, cancer and cardiovascular disease. Here, we demonstrate that the p53 family transcription factor, TAp73, contributes to the oxidative stress response by participating in the control of protein synthesis. Regulation of mRNA translation ensures a prompt and efficient method to overcome stress and TAp73 depletion results in aberrant ribosomal biogenesis and impaired protein synthesis. In particular, TAp73 is important for maintaining active translation of mitochondrial transcripts in response to oxidative stress, thus promoting mitochondrial activity that contributes to adaptation to stress conditions. Our data therefore reveal a novel, unexpected role for TAp73 in regulating protein synthesis responsible for its homeostatic ability.

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INTRODUCTION

TAp73 is a member of the p53 family of transcription factors that has important functions in development (1), reproduction (2, 3) and cancer (4, 5). TAp73 plays a key role in aging, as TAp73$^{-/-}$ mice exhibit accelerated aging, oxidative stress and mitochondrial defects that are attributed to insufficient expression of the mitochondrial protein cytocrome-C-oxydatese-subunit-4 (COX4i1), a direct TAp73 target gene (6). Furthermore, TAp73 promotes the expression of glyceraldehyde-6-phosphate dehydrogenase (G6PD) and glutaminase-2 (GLS-2), thus stimulating anti-oxidant and biosynthetic pathways (3, 7, 8). In cancer, TAp73 functions as a tumour suppressor, acting both in the maintenance of genomic stability (3) and opposes angiogenesis by promoting HIF-1α degradation (5, 9). This evidence implicates TAp73 as an important factor for maintaining cellular homeostasis and reinforces its role as a tumour suppressor.

Regulation of protein synthesis allows cells to exert tight control on transcripts translated under defined stress conditions (10). A master regulator of these processes is the mammalian target of rapamycin (mTOR) kinase, which coordinates the cellular responses to a range of cell stresses including amino acid deprivation and oxidative stress (11). Importantly, mTOR regulates both the global and selective translation of transcripts (12). Translation is also regulated at the elongation stage by the eukaryotic elongation factor 2 kinase (eEF2K), which directly phosphorylates its substrate, eukaryotic elongation factor 2 (eEF2), thereby inhibiting translation elongation (13). Like mTOR, eEF2K is also known to promote the translation of specific transcripts (14). The eEF2K/eEF2 axis is involved in reducing translation elongation rates following ribosomal stress and dysfunctional ribosome biogenesis (15, 16), eEF2K is indeed downregulated as compensatory mechanism in cells that exhibit defective ribosomal RNA (rRNA) processing (17).

Although crosstalk between mTOR and TAp73 has been recently reported (18) to regulate autophagy, a direct role for TAp73 in translational regulation has never been addressed. Here, we unravel a new mechanism involving mRNA translation by which TAp73 contributes to the oxidative stress response. TAp73 depletion results in aberrant rRNA processing, reduced translation elongation and impaired protein synthesis. Concurrently, TAp73 knockdown increases the cellular sensitivity to oxidative stress that correlates with reduced ATP levels, hyperactivation of 5' adenosine monophosphate-activated protein kinase (AMPK) and translational defects. TAp73 maintains mitochondrial function by acting as a regulator of the translation of selected mRNAs encoding mitochondrial proteins. Therefore, the transcription factor TAp73 plays an important role in regulating mRNA translation, thus contributing to the oxidative stress response.

RESULTS

TAp73 depletion results in reduced tolerance to oxidative stress and impaired protein synthesis

We used two different siRNAs specific for the TA isoform to knockdown (KD) TAp73 expression in HEK293T cells to investigate the role of this transcription factor in the oxidative stress response (Fig. 1A, 1B). This cell line expresses high levels of TAp73, with barely detectable levels of the ΔN isoform (SI Appendix, Fig. 1A) and equally importantly, has high expression of the Simian Vacuolating Virus 40 Tag protein which inactivates p53 (19). TAp73 KD cells accumulated more reactive oxygen species (ROS) than control cells following treatment with different concentrations of H_2O_2 (150μM and 300μM) or sub-lethal dose of the superoxide-inducer Doxorubicin (Figure 1A,B and SI Appendix, Fig. S1B, S2B-D), and displayed increased cell death following exposure to H_2O_2 (Fig. 1C and SI Appendix, Fig. S2A). During stress, such as an oxidative imbalance, translation regulation occupies a central

position in restoring cellular homeostasis (10, 11), reducing overall protein synthesis and promoting the translation of a specific subset of mRNAs (20). We pulsed TAp73 KD and control cells with ^{35}S-labelled methionine and cysteine and measured their incorporation in *de novo* synthesised proteins to explore a possible effect of TAp73 on protein synthesis. TAp73-depleted cells exhibited reduced protein synthesis (Fig. 1D, SI Appendix, Fig. S3A), which was particularly prominent under oxidative stress conditions (150µM H_2O_2 for 3 hours). As expected, after H_2O_2 treatment, a decrease in protein synthesis rate was also observed in control cells (10). We previously reported (6) that TAp73 knockout MEFs exhibit increased intracellular basal ROS. However, ROS-scavenger N-acetyl cysteine (NAC) treatment did not restore protein synthesis in TAp73 KD cells (SI Appendix, Fig. S3B), thus ruling out the possibility that the protein synthesis reduction was not caused by increased basal ROS. We then examined the distribution of RNAs using sucrose density gradient ultracentrifugation, to separate translating ribosomes (polysomes) from non-translating fractions that contain the dissociated ribosomal subunits (sub-polysomes). Consistent with the decrease in protein synthesis rates, we observed a relatively small reduction in the number of polysomes in TAp73 KD cells under resting conditions (Fig. 1E, top panel, and SI Appendix, Fig. S3C). The H_2O_2 treatment reduced the polysomal/sub-polysomal ratio in control cells (SI Appendix, Fig. 3D) and induced a decrease in the number of polysomes in TAp73 KD cells (Fig. 1E, bottom panel), indicating a substantial translational block when cells were challenged with pro-oxidant agents in the absence of TAp73. Together, our data reveal an unexpected role for TAp73 in regulating protein synthesis and suggest that this role is important in the oxidative stress response.

TAp73 regulates translation elongation

We next asked how TAp73 affects protein synthesis. Despite the large decrease in the global translation rate, we only observed a marginal reduction in the amount of polysomes in TAp73 KD cells (Fig. 1E). These profiles appeared to be greatly different from cells in which translation initiation is impaired (e.g. during apoptosis or genotoxic stress, (21, 22). Indeed, in this case, the polysomal fraction is considerably reduced and is associated with a concomitant large increase in the amount of sub-polysomes. Based on these observations, we noticed that mRNAs were retained in the polysomal fraction of TAp73 KD cells, implying a possible translation elongation defect. Thus, we assessed whether TAp73 preferentially affected translation elongation or initiation by evaluating the expression and phosphorylation of eEF2 and its kinase eEF2K, in addition to the initiation factors 4EBP1 and eIF4E. Consistent with the results shown in Fig. 1E, we observed an increase in the inhibitory phosphorylation of eEF2 in TAp73 KD cells compared to control cells, thus strongly suggesting an elongation blockade (Fig. 2A, SI Appendix, Fig. S4A,B). Consistent with this evidence, inhibitory phosphorylation of eEF2K was reduced following TAp73 depletion (Fig. 2A). We also confirmed these data in the H1299 p53-null lung cancer cell line (Fig. 2B). Moreover, we observed a reduction in the activity of the mTOR pathway in TAp73 KD cells, as demonstrated by reduced p70S6K and S6 phosphorylation (Fig. 2A, SI Appendix, Fig. S4A). Consistently the m^7-GTP pull down of the eIF4F cap-binding complex (23) did not reveal any disruption in the assembly after TAp73 KD (SI Appendix, Fig. S5A). Furthermore, TAp73 depletion did not lead to any changes in eIF2α phosphorylation, suggesting that the endoplasmic reticulum (ER) stress response is not influenced by TAp73 (Fig. 2A,B). Taken together, these data strongly imply an involvement of translation elongation blockade in reducing global protein synthesis after TAp73 KD. We measured the relative rate of translation elongation in TAp73 KD and control cells using the harringtonine run-off assay to validate this hypothesis. Harringtonine blocks translation initiation, allowing only ribosomes committed to elongation to terminate their mRNAs while preventing re-initiation; cycloheximide (CHX), which blocks elongation, is then added at different time points and the polysome abundance is calculated following sucrose gradient ultracentrifugation of lysates.

An increased amount of polysomes remaining at 90 s and 120 s before CHX addition was observed in TAp73 KD cells compared to control cells, confirming a reduction in the rate of elongation (Fig. 2E and SI Appendix, Fig. S5B). Overall, our results reveal an important role for TAp73 in promoting translation elongation.

TAp73 supports the translation of ribosomal biogenesis factors

Both defective rRNA processing and ribosomal stress inhibit translation elongation. We asked whether TAp73 is involved in ribosome biogenesis to further explore the mechanisms underlying the TAp73-dependent translation elongation defects. Therefore, we examined the ribosome profiles, which showed a significant reduction in the 40S:60S ratio following TAp73 KD, indicative of a defect in ribosome assembly (Fig. 3A). We performed a microarray analysis of total RNA from TAp73 KD to assess the involvement of TAp73 in this process. As expected, TAp73 KD resulted in a massive change in the cellular transcriptome (SI Appendix, Dataset S1, SI Appendix, Fig. S8A). However, the gene ontology (GO) analysis did not reveal changes that explained our observations, suggesting that the observed effect did not directly involve transcriptional modifications. We therefore asked whether changes in the cellular translatome might account for the ribosome assembly defect. Thus, we used RNAs collected from sub- and polysomal fractions of control and TAp73 KD cells and a microarray approach. TAp73-dependent changes in the translatome were observed following TAp73 KD (SI Appendix, Dataset S2, Fig. S8B,C). GO analysis revealed several categories that were enriched in the list of transcripts that exhibited a reduction in polysomal distribution, of which the top category was "Nucleolus" (Fig. 3B). One of the major functions of the nucleolus is rRNA processing, therefore, we hypothesised that TAp73 may play a role in this process. Several transcripts whose proteins are known to play a role in rRNA processing and biogenesis, including UTP18, XPO1, DKC1 and NOL9 exhibited reduced expression in polysomes, indicating a reduction in their translation (SI Appendix, Dataset S2, Fig. S6A). We focused on UTP18 and XPO1, both critical factors in ribosome biogenesis. UTP18 is an important component of the small subunit processome involved in the nucleolar processing of the pre-18S rRNA, while XPO1 is the nuclear pre-40S exportin. We observed decreased expression both of UTP18 and XPO1 in TAp73 depleted cells (Fig. 3C). The KD of these factors was able to recapitulate the reduction in global translation rate (Fig. 3D) and was associated with eEF2 phosphorylation. Furthermore, the simultaneous depletion of eEF2K and TAp73 did not restore the expression of UTP18, thus demonstrating that the elongation block is a consequence and not a cause of the defect in rRNA processing and ribosome assembly (Fig. 3E, SI Appendix, Fig. S6B).

We then analysed rRNA precursors in TAp73-depleted and control cells by northern blotting to examine the defect in rRNA processing. Consistent with our hypothesis, we observed impaired rRNA processing in TAp73 KD cells, as evidenced by a 5-fold increase in the earliest rRNA precursors (45S/41S), which were detected using two independent probes (Fig. 3F). An increase was also observed for other pre-rRNA species upstream of 18S, 5.8S and 28S RNA (30S, 21S, 18SE, 32S, and 12S), although the difference was not significant. Taken together, these data reveal an unknown function of TAp73 in promoting translation by facilitating the association of nucleolar protein-encoding transcripts with polysomes.

TAp73 supports mitochondrial activity under oxidative stress conditions

We previously showed that TAp73 depleted cells exhibit increased sensitivity to oxidative stress (Fig. 1). A greater accumulation of ROS following H_2O_2 or Doxorubicin treatments was also detectable in mitochondria of TAp73 KD cells, where increased levels of superoxide were observed (SI Appendix, Fig. S2C and 7A). Since defects in oxidative phosphorylation elicit oxidative stress *in vitro* (24) (6), we therefore asked whether the reduced tolerance to oxidative stress is associated to defects in mitochondrial activity. TAp73 depletion did not affect oxygen consumption under resting conditions, however, a reduction

was observed in TAp73 depleted cells under oxidative stress (SI Appendix, Fig. S7B). Importantly, following rotenone-succinate stimulation, no differences were observed in oxygen consumption between TAp73 KD and control cells, suggesting a defect in mitochondrial complex I (SI Appendix, Fig. S7B).

Consistent with the oxygen consumption analysis, we observed a greater decrease in ATP levels in the TAp73 KD cells than in control cells following the H_2O_2 treatment (Fig. 4A). Based on these data, we conclude that TAp73 supports mitochondrial activity and energy production under oxidative stress conditions.

TAp73 influences the mTOR pathway and eEF2-mediated protein synthesis under oxidative stress conditions

One important arm of the ATP sensor, AMPK, is the mTOR pathway, of which AMPK is a negative regulator. Because TAp73 depletion resulted in accelerated reduction of ATP levels in cells treated with H_2O_2, this change might be reflected in a more robust activation of AMPK and phosphorylation of its downstream targets. Indeed, following H_2O_2 treatment TAp73 KD cells exhibited a more robust activation of AMPK than control cells, as reflected by AMPK phosphorylation and ACC phosphorylation, which lasted for a longer time (Fig. 4B, SI Appendix, Fig. S4D). Moreover, a strong reduction in the activity of the mTOR pathway was observed in the TAp73 KD cells, as reflected in the phosphorylation status of two major effectors of the mTOR pathway, S6 and 4EBP1 (Fig. 4B, SI Appendix, Fig. S4C,D). Thus, TAp73 is important for the ability of the cell to sustain the activation of the mTOR pathway and reduce the activation of AMPK under oxidative stress conditions. AMPK is known to negatively regulate translation elongation by activating eEF2K (13, 14). Following the H_2O_2 treatment, eEF2 phosphorylation was increased in TAp73-depleted cells compared to control cells, as the increase was much more substantial than the level observed under basal conditions (Fig. 4B, 300 min in SI Appendix, Fig. S4D). These results showed the ability of TAp73 to control translation elongation via AMPK, even under oxidative stress conditions. Energetic stress also triggers the ER stress response and eIF2α phosphorylation (25), resulting in the inhibition of translation initiation. We observed a rapid increase in eIF2α phosphorylation in both TAp73-depleted and control cells following H_2O_2 exposure, and this signal persisted for the duration of the experiment (Fig. 4B). However, there were no observable differences in the eIF2α response. Thus, we concluded that this arm of the stress response is not sensitive to TAp73 depletion and that translation initiation downstream of eIF2α is blocked by H_2O_2 independent of TAp73. Together, mTOR inhibition, eIF2α phosphorylation or eEF2 hyper-phosphorylation led to a massive block of translation and a reduction in the global protein synthesis rate (Fig. 1).

TAp73 regulates the translatome under oxidative stress conditions

According to our results, TAp73 influences the cellular response to pro-oxidant insults. In particular, under oxidative stress conditions, TAp73 expression supports mitochondrial activity, ATP synthesis and the activation of the mTOR pathway. Analysis of the total RNA from cells +/- TAp73 and +/- H_2O_2 (3h, 150 mM; sub-lethal dose) revealed that TAp73 KD resulted in a significant change of cellular transcriptome under resting conditions, however, H_2O_2 treatment did not cause any significant transcriptional changes (SI Appendix, Fig. S8). This suggests that the cellular response to H_2O_2, under these conditions, does not involve transcriptional changes and perhaps, TAp73 contribution to redox response is transcriptional independent.

Hence, we asked whether the sensitivity of the TAp73-depleted cells could be explained by their altered translational programme in response to the H_2O_2 treatment. Using polysome profiling and a microarray analysis of H_2O_2-treated cells, we determined that a significant number of transcripts related to mitochondria were lost from the polysomal fraction in the TAp73 KD cells under oxidative stress conditions (Fig. 4C, SI Appendix, Dataset S2,

Fig. S8). In agreement with the reduced mitochondrial activity (SI Appendix, Fig. S7B), we observed that mRNAs for the mitochondrial complex I subunits NDUFB8, NDUFB9, NDUFS3 and NDUFS8 lost their polysomal distribution in TAp73 KD cells following the H_2O_2 treatment (Fig. 4D). Thus, TAp73 may play an important role in protecting mitochondria from oxidative stress by facilitating the translation of mitochondrial proteins under these conditions. To examine further the link between defective translation and reduced tolerance to oxidative stress, we measured ROS in cells depleted for rRNA biogenesis factors, UTP18 and XPO1, following H_2O_2 treatment. Our data show that KD of either or both was associated with increased accumulation of ROS compared to control, thus suggesting that the impaired translational machinery resulting from TAp73 depletion underpinned the defective response to oxidative stress (Fig. 4E). Based on these data, we conclude that TAp73 plays an important role in the translation of mitochondrial proteins during oxidative stress, thus promoting mitochondrial activity and coupling the latter to cellular pathways regulating translation. Overall, TAp73 promotes protein translation under oxidative stress conditions, sustaining mitochondrial activity and thus enhancing the capability of the cells to cope with redox insults (SI Appendix, Fig. S9).

DISCUSSION

The transcription factor TAp73 is a p53 family member known to function as an important homeostasis-maintaining component in the context of cellular metabolism and oxidative stress (6, 8) by acting as a transcriptional regulator of several genes. Here we demonstrate that TAp73 contributes to cellular homeostasis and supports the cells abilities to cope with oxidative stress by impacting protein synthesis.

Using ribosome profiling, we found that TAp73 expression is necessary to retain nucleolus-specific transcripts in the polysomal fraction. Thus, TAp73 depletion resulted in aberrant synthesis of nucleolar proteins and defective rRNA processing. The mechanism by which TAp73 affects this process is unknown and we did not identify any direct involvement of transcription. This implies that further investigation will be necessary to fully understand the role of TAp73 in controlling the cellular translatome.

How does TAp73 promote protein synthesis? As aberrant rRNA processing leads to defective ribosomal assembly and reduced translation, TAp73 plausibly promotes protein synthesis by facilitating the translation of nucleolar proteins. In agreement with recent studies (15, 16, 26), we report that defective rRNA processing resulted in increased eEF2 phosphorylation and reduced translation elongation. Furthermore, depletion of two TAp73 target transcripts (UTP18, XPO1) recapitulated TAp73-dependent effects on the translation elongation pathway and global protein synthesis. Thus reduced expression of ribosome biogenesis factors, such UTP18, appears to be a primary defect upstream of elongation block, supported by our data obtained following by the knock down eEF2K. In this regard, the suppression of translational elongation is a key part of the cellular response to ribosomal stress. We ruled out an involvement of basal ROS accumulation produced by TAp73 depletion on the rate of mRNA translation: treatment with the anti-oxidant NAC did not revert the decrease in protein synthesis rates.

Notably, the effects observed for TAp73 on protein synthesis and translation regulation pathways are specific for this member of p53 family and are different from those of p53. The latter has been described as a repressor of global protein synthesis (27), showing inhibitory effects on translation initiation and ribosome biogenesis. In this regard, our data consistently indicate that p53 depletion results in a slight increase in protein synthesis rates.

The loss of TAp73 leads to defective ribosomes and inefficient protein synthesis that could alter the cellular capability to promptly respond to stress, such as ROS. Indeed, analysis of the translatome during oxidative stress reveals a significant shift in mitochondrial protein-encoding transcripts to the sub-polysomal fraction, thus impairing mitochondrial activity in TAp73 KD cells. As functional mitochondria are necessary for the cellular response

to oxidative damage (6) (24), it is reasonable to conclude that corrupted mitochondrial metabolism may be responsible for the reduced tolerance to redox imbalance in TAp73 depleted cells. Importantly, our data show that the loss of rRNA biogenesis factors (UTP18, XPO1) sensitises cells to oxidative stress, as demonstrated by the accumulation of more ROS under H_2O_2 treatment. Although this approach does not fully recapitulate the complexity of TAp73 knockdown in terms of regulated genes and cell response, these data highlight the fact that fully functional ribosomes are required to maintain oxidative balance. At this stage, it is difficult to speculate how defective ribosomes, downstream of TAp73 loss, impair the translation of a specific subset of transcripts. In this regard, it is very tempting to propose a mechanism involving both the specificity in 5'-UTR sequences (28) and mechanisms of surveillance of mRNA translation (29, 30), with the latter able to recognize and dissociate defective/stalled ribosomes along the mRNA with consequent redistribution of transcripts.

TAp73 depletion reduces ATP levels under oxidative stress possibly explaining the activation of AMPK. The robust up-regulation of the AMPK pathway in TAp73 depleted cells is associated with a sustained down-regulation of its downstream target mTOR, a reduction that correlates with strong repression of the protein synthesis rate. Moreover, the 4EBP1 arm of the mTOR pathway exhibited the greatest alterations in TAp73 KD cells under oxidative stress conditions. Interestingly, signalling through this branch of the mTOR pathway is also responsible for selective mRNA translation promoted by mTOR (12).

The inability to maintain an appropriate redox homeostasis in cells depleted of TAp73 results in cell death when they are exposed to high doses of hydrogen peroxide and for long periods. Toxic levels of ROS activate a caspase-independent cell death, defined "oxeiptosis" (31). Although we did not investigate the mechanism that drives cell death, it is possible that the analogous molecular events also lead to death in our model.

ROS can mediate "non-specific" damage to DNA with consequent mutations and higher probability of oncogenic events. In this context our findings indicate a novel mechanism mediated by mRNA translation that might be critical for the well-known function of TAp73 as a tumour suppressor (3) .

In summary, our work reveals an unexplored role for the transcription factor TAp73 in regulating key pathways linked to mRNA translation. We present evidence that TAp73 promotes protein synthesis, possibly by facilitating the translation of transcripts involved in rRNA processing. Moreover, under oxidative stress conditions, TAp73 supports a specific translational programme required to maintain mitochondrial activity, helping cells to cope with redox stress. In addition to the well-established transcriptional role of TAp73 in maintaining the oxidative balance, our work describes a new mechanism for TAp73-dependent responses to oxidative stress involving regulation of protein synthesis (SI Appendix, Fig. S9).

MATERIALS AND METHODS
Cell culture
HEK293T and A549 cells were cultured in DMEM (Gibco) supplemented with 10% FBS (Sigma-Aldrich) and penicillin/streptomycin (1U mL^{-1}) (Gibco). H1299 cells were maintained in RPMI 1640 medium (Gibco) supplemented with 10% FCS and penicillin/streptomycin (1 U mL^{-1}).

Determination of protein synthesis rates
Cells in methionine-cysteine-free medium (Sigma-Aldrich) were pulsed with 1.11 MBq/ml [^{35}S]-labelled methionine-cysteine (Hartman Analytical) for 30 min, washed with PBS, lysed in PLB buffer (Promega) and then trichloroacetic acid (TCA) was added. Insoluble proteins were captured on glass microfiber filter papers (Whatmann), and radioactivity was determined by scintillation counting (National Diagnostics) and normalized to the total protein content.

Mitochondrial activity

Cells were transfected with siRNAs for 72 hours and then treated with 150μM H_2O_2 for 3 hours. Cell pellets were collected and respiration was measured using Oroboros, as previously described (6).

Full Matherial and Methods are available in supplementary information.

ACKNOWLEDGMENTS

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FIGURE LEGENDS

Figure 1. TAp73 KD affects the oxidative stress response and protein synthesis. A,B. HEK293T cells were transfected with siRNAs targeting TAp73 or scrambled control for 72h and then were challenged with 300µM or 150µM H_2O_2. Cells were washed and incubated in the presence of CM-DCFDA for 30 min and then analysed by flow cytometry. % of cells with high DCFDA is shown (mean ± SD, n=3; *p<0.05 vs Scr). KD efficiency was tested (top panels). **C.** Cell death was determined using PI staining and flow cytometry (300µM H_2O_2 for

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8 hours) (mean ± SD, n=3; *p<0.05 vs Scr). **D.** ^{35}S-methionine and cysteine incorporation in the proteome was measured under control conditions and after 150μM H$_2$O$_2$ treatment for 3 hours (mean ± SD, n=3; *p<0.05 vs Scr). **E.** Representative polysome profiles obtained by sucrose-density ultracentrifugation of HEK293T cell lysates transfected as in **A**. Top panel: control conditions. Bottom panel: cells were treated with 150μM H$_2$O$_2$ for 3h after silencing.

Figure 2. TAp73 KD impairs translation elongation. A. Immunoblot analysis of lysates obtained from HEK293T cells transfected with the indicated siRNAs for 72h. **B.** Immunoblot analysis of lysates obtained from H1299 cells transfected with the indicated siRNAs for 48h. **C.** ^{35}S-methionine and cysteine incorporation in A549 (p53wt) cells transfected with siRNA targeting p53 or scrambled control for 72h (mean ± SD, n=3, *p<0.05 vs Scr). KD efficiency was tested (top panel). **D.** Immunoblot analysis of lysates obtained from A549 cells transfected as in **C. E.** Ribosome run-off assay performed in HEK293T cells transfected as in **A** (mean ± SD, n=3; *p<0.05 vs Scr 90sec and vs Scr 120sec, respectively).

Figure 3. TAp73 depletion results in aberrant rRNA processing. A. 40S:60S ratio in polysome profiles of HEK293T cells transfected with the indicated siRNAs for 72h (mean ± SD, n=4, *p<0.05). **B.** GO analysis of transcripts that exhibit reduction on polysomes following TAp73 KD (Dataset S2). **C.** Immunoblot analysis of UTP18 and XPO1 in lysates from HEK293T cells transfected as in **A** (star indicates nonspecific band). **D.** ^{35}S-methionine and cysteine incorporation in HEK293T cells transfected with siRNAs targeting ribosome biogenesis factors for 72h (mean ± SD, n=3, *p<0.05 vs Scr) (star in blot analysis indicates nonspecific band). **E.** Immunoblot analysis of double knock down experiment in lysates obtained from HEK293T transfected with the indicated siRNAs (star indicates nonspecific band). **F.** Total RNAs of HEK293T cells transfected as in A were size resolved and used for Northern blotting. Radiolabelled oligonucleotide probes revealed the abundance of small (ITS1) and large (ITS2) ribosomal subunit rRNAs precursors and the mature 28S, 18S and 5.8S rRNAs (top panel). Each lane is a biological replicate. Note that the 45S/41S species is a precursor of both small and large subunits. The abundance of each rRNA species was quantified by densitometry (bottom panel) (mean ± SD, n=3, p-values are shown in the figure).

Figure 4. TAp73 depletion results in loss of ATP, activation of the AMPK pathway and inhibition of mTOR under oxidative stress. A. HEK293T cells were transfected with siRNA targeting TAp73 or scrambled control for 72h and ATP levels were measured after treatment with H$_2$O$_2$ as indicated (mean ± SD, n=3; *p<0.05 vs Scr). **B.** Lysates obtained from HEK293T cells transfected with the indicated siRNAs and treated with 150μm H$_2$O$_2$ for the indicated time points were analyzed by immunoblot using the indicated antibodies. **C.** GO analysis of transcripts that exhibit reduction on polysomes following TAp73 KD and treatment with 150μM H$_2$O$_2$ for 3 hours (SI Appendix, Dateset S2). **D.** HEK293T cells were transfected with the indicated siRNAs for 72h. Sucrose-density ultracentrifugation was performed following treatment with 150μM H$_2$O$_2$ for 3 hours and sub-polysomal and polysomal fractions were collected. mRNAs levels were measured in each fraction by RT-qPCR (mean ± SD, n=3). **E.** ROS analysis in HEK293T cells transfected with siRNAs targeting ribosome biogenesis factors for 72h and then treated with 150μM H$_2$O$_2$ for 3h (mean ± SD, n=3, *p<0.05).