Single Cell Biology
ISSN: 2168-9431

Jiang and Chen, Single Cell Biol 2016, 5:4
DOI: 10.4172/2168-9431.1000154

Commentary Open Access

Δ 133p53 Functions to Maintain Redox Homeostasis in Response to Low ROS Stresses

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Rec date: Dec 16, 2016; **Acc date:** Dec 29, 2016; **Pub date:** Dec 31, 2016

Abstract

Reactive oxygen species (ROS) can serve as intracellular signals that promote cell proliferation and survival, or as toxicants that cause abnormal cell death and senescence. Tumour repressor p53 is a ROS-active transcription factor that upregulates the expression of antioxidant genes during low oxidative stresses, but promotes the expression of pro-oxidative and apoptotic genes during high level stresses. The underlying mechanisms for p53 selectively to transcribe different groups of genes remain elusive. We recently found that p53 isoform Δ133p53 is strongly induced by a low concentration of H_2O_2 (50 μM), as opposed to higher concentrations, and functions to promote cell survival. Under the low oxidative stress, Δ133p53 is required for p53 to selectively upregulate the transcription of the antioxidant genes SESN1 and SOD1 by binding to their promoters. The knockdown of either p53 or Δ133p53 in low oxidative stresses increases the intracellular $O_2^{\bullet-}$ level, which results in accumulation of DNA damage, cell growth arrest at the G2 phase that in turn leads to enhanced cell senescence. Our findings suggest that an induction of Δ133p53 may correlate with ageing and human pathologies associated with oxidative stresses.

Keywords: ROS; p53; Δ133p53; Antioxidant gene

Commentary

Reactive oxygen species (ROS) including superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$) and non-radical species hydrogen peroxide (H_2O_2) are generated during mitochondrial oxidative metabolism and as a cellular response to xenobiotics and bacterial invasion in aerobic organisms [1,2]. Moderate levels of ROS can function as signals that promote cell growth and division [3-5]. However, when overproduced, ROS overwhelm a cell's capacity to maintain redox homeostasis, and can cause oxidative stress, which results in the oxidation of macromolecules such as proteins, membrane lipids and mitochondria or genomic DNA [6,7]. The detrimental accumulation of ROS eventually leads to abnormal cell death and senescence, which contributes to the development of neurodegenerative diseases, cancer, and aging-related pathologies [8,9].

To maintain redox homeostasis, organisms have evolved with numerous endogenous antioxidant defense systems including both enzymatic and non-enzymatic antioxidant mechanisms that can either scavenge ROS or prevent their formation [10]. Tumour repressor p53 plays important and complex roles in response to oxidative stress [11-14]. In physiological and low levels of oxidative stress conditions, p53 promotes cell survival by triggering the expression of antioxidant genes such as superoxide dismutase 1 (SOD1), superoxide dismutase 2 (SOD_2), glutathione peroxidase 1 (GPX1), Sestrin 1 (SESN1), Sestrin 2 (SESN2) and aldehyde dehydrogenase 4 family members A1 (ALDH4A1), which restore oxidative homeostasis [15-20]. In contrast, in response to high levels of oxidative stress p53 induces apoptosis by upregulating the expression of pro-oxidative genes such as PIG3 and proline oxidase, and apoptotic genes such as BAX and PUMA [18,21-29]. However, how p53 triggers the expression of different groups of genes in response to various levels of ROS remains perplexing until our recent article entitled "p53 coordinates with Δ133p53 isoform to promote cell survival under low-level oxidative stress" was published. Δ133p53 is an N-terminal truncated form of p53 with the deletion of both the MDM2-interacting motif and the transcription activation domain, together with partial deletion of the DNA-binding domain [30,31]. Δ133p53 is transcribed by an alternative p53 promoter located in intron 4 of the p53 gene [32-34]. Full-length p53 can directly transactivate its transcription in response to both developmental and DNA damage stresses. The induction of Δ133p53 subsequently antagonizes p53-mediated apoptosis [30,31,34]. However, the basal expression level of Δ133p53 can inhibit p53-mediated replicative senescence by downregulating the expression of $p21^{WAF1}$ and miR-34a in normal human fibroblasts [35]. Being p53 target, Δ133p53 was strongly induced only by γ-irradiation, but not ultraviolet (UV) irradiation or heat shock treatment, whereas full-length p53 was activated under all three challenges. In response to γ-irradiation, Δ133p53 represses cell apoptosis and promotes DNA DSB repair via upregulating the transcription of repair genes [36]. Therefore, it is of interest to know whether Δ133p53 plays a role in response to ROS stresses.

In our recent study, we used H_2O_2, a model oxidant, to explore the biological function of Δ133p53 in human cells upon oxidative stresses [37]. We found that the induction of p53 protein and transcript by H_2O_2 was dose-dependent within the concentrations tested (25 μM to 400 μM). However, the increase of Δ133p53 protein and transcript appeared to be limited to the lower dose range, with a maximum induction at 50 μM H_2O_2, followed by a gradual drop at latter concentrations. Interestingly, H_2O_2-induced cell survival response correlated nicely to the level of Δ133p53 expression. Using various cell viability analysis methods including MTT, WST-8, Trypan blue staining and BRDU incorporation, we showed that an overexpression of Δ133p53 augmented, whereas an under expression removed the 50 μM H_2O_2-induced increase in cell viability. The pro-survival role of

Δ133p53 in response to low ROS stresses was confirmed in this study with different cell lines and another oxidant, menadione (vitamin K3).

To investigate whether this role is associated with the protein anti-apoptotic activity, we performed FACS analysis using anti-Annexin V antibody staining. Our data revealed that neither the knockdown nor overexpression of Δ133p53 produced an obvious effect on cell apoptosis under 50 μM H_2O_2 treatment. On the other hand, cell cycle analysis with Propidium Iodide (PI) staining revealed that the proportion of cells at the G2 phase was significantly increased by the knockdown of Δ133p53 under the same treatment. These results demonstrated that under 50 μM H_2O_2 treatment, Δ133p53 increases cell viability by promoting cell division, instead of exerting its anti-apoptotic activity.

Dihydroethidium (DHE) staining analysis uncovered that the knockdown of Δ133p53 significantly increased intracellular $O_2^{\bullet-}$ level upon 50 μM H_2O_2 treatment. Comet assay showed that the increased accumulation of ROS induced DNA damage with single-stranded breaks (SSB), instead of DNA double-stranded breaks (DSB). The accumulation of DNA SSBs from the knockdown of Δ133p53 demonstrated that Δ133p53's positive role in DNA DSB repair does not play a role in promoting cell survival during low ROS stresses. Eventually, a high-level DNA damage brings about cell growth arrest at G2 phase which finally leads to cell senescence.

In our study of the underlying molecular mechanisms, we found that Δ133p53 upregulated the transcription of the antioxidant genes SESN1 and SOD1 in a p53 dependent manner. Furthermore, Δ133p53 was required for p53 to increase the expression of these two genes in response to low oxidative stress. Therefore, our study revealed that p53 coordinates its isoform Δ133p53 to selectively transactivate the expression of antioxidant genes to promote cell survival in low oxidative stress conditions.

A number of questions remain unanswered. For instance, why does the expression of Δ133p53 gradually decrease with the concentration of H_2O_2 increases beyond 50 μM? How does Δ133p53 mediate p53 to increase the transcription of antioxidant genes? In addition, it has been well-established that increases in ROS levels and decreases in antioxidant capacity contribute to the ageing process through the oxidation of different macromolecules, such as lipids, proteins and genomic or mitochondria DNA [1]. The protein p53 has also been linked to ageing [12]. For instance, the overexpression of Δ40p53 (N-terminal truncated isoform) in mice results in increased p53 activity and leads to accelerated ageing [38]. However, mice carrying both an additional copy of genomic p53 (including all its isoforms) and ARF loci exhibit an increased expression of antioxidant activity and decreased levels of endogenous oxidative stresses, which are both correlated with enhanced life span [39]. These results suggested possible roles of the other p53 isoforms in this phenomenon. Here, we showed that Δ133p53 is required for p53 to upregulate the expression of antioxidant genes in response to low oxidative stress. It will be interesting to know whether the p53 isoform Δ133p53 plays a role in ageing process. These questions deserve further explorations.

In summary, we propose a hypothetical model for a dual role of p53 in response to ROS stress in Figure 1. In response to low oxidative stresses (under a certain threshold), p53 is accumulated to a relative low level for transcription of Δ133p53. Subsequently, Δ133p53 coordinates with p53 to promote cell survival by upregulating expression of antioxidant genes; whereas, in high oxidative stress conditions (beyond a certain threshold), p53 is accumulated to a high level with less Δ133p53 induction. Higher level p53 induces cell death by upregulating expression of pro-oxidative and apoptotic genes.

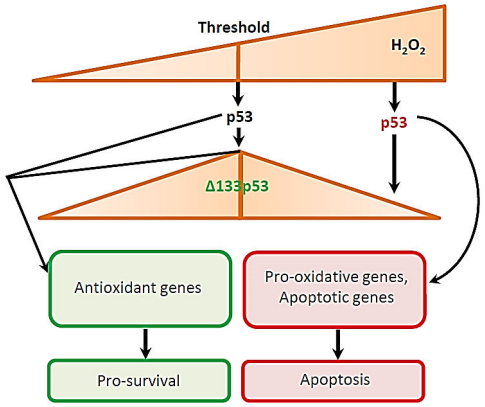

Figure 1: p53 signaling in response to oxidative stresses. Upon low oxidative stresses (under a certain threshold), p53 protein is activated to a relative low level for transcription of its target genes including Δ133p53. The expression of Δ133p53 can coordinate with p53 to increase the expression of antioxidant genes such as: SOD1 and SEN1. Subsequently, the expression of SOD1 and SEN1promotes cell survival by maintaining redox homeostasis; Under high oxidative stresses (beyond a certain threshold), p53 protein is accumulated to a high level to guide cells to apoptosis by inducing the expression of pro-oxidative and apoptotic genes.

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