

IN THE SPOTLIGHT

Spatiofunctional Dynamics of NKX3.1 to Safeguard the Prostate from Cancer

Andrew J. Finch¹ and Esther Baena^{2,3}

Summary: A novel role of NKX3.1 in the mitochondria regulating the transcription of the electron transport chain components is reported. Mechanistically, HSPA9 chaperones NKX3.1 into the mitochondria in response to oxidative stress to regulate reactive oxygen species and suppress tumor initiation.

See related article by Papachristodoulou et al., p. 2316 (6).

Prostate cancer is the most common cancer in males. This is in part due to the high susceptibility of the prostate gland to DNA damage caused by inflammation and oxidative stress. *NKX3.1* alterations are among the earliest genetic events in prostate cancer, and its deficiency has been associated with oxidative stress (1, 2) and DNA damage (3). Previous studies using mouse models have demonstrated its role in prostate differentiation and luminal progenitor cells (4, 5), all functions so far assigned to transcriptional regulation in the nucleus.

Here, Papachristodoulou and colleagues demonstrate a novel non-nuclear role for NKX3.1 in prostate cells (6). The authors show a direct link between NKX3.1 transient mitochondrial localization in response to oxidative stress and the transcriptional regulation of mitochondrial electron transport chain components (Fig. 1). To dissect the underlying mechanism, the authors used chemically induced chronic oxidative stress to assess the consequences of reactive oxygen species (ROS) production in the presence or absence of NKX3.1 in prostate tissue. Furthermore, they showed very elegantly the protective role of NKX3.1 against oxidative stress, which is impaired in NKX3.1 cancer-associated variants (R52C or T164A) that are unable to translocate to the mitochondria or bind mitochondrial DNA, respectively. Analysis of the interactors of NKX3.1 in oxidative stress conditions revealed HSPA9 as a candidate chaperone responsible for its translocation to the mitochondria, and its absence impaired NKX3.1 mitochondrial localization. Notably, the NKX3.1 translocated to the mitochondria does not translocate out of the nucleus upon oxidative stress, as the nuclear pool is maintained. Thus, the mitochondrial function of NKX3.1

is completely separate from its nuclear function. Overall, the findings on the mitochondrial function of NKX3.1 are novel and interesting and propose a completely new “regulation paradigm” of cellular homeostasis that can be used for patient stratification and prevention of aggressive disease. As with the description of any novel biological mechanism, this work raises several other interesting questions.

The interdependency of the mitochondrion and the rest of the cell is a paradigm of endosymbiosis, even to the extent that the master regulator of mitochondrial biogenesis, PGC1 α (peroxisome proliferator-activated receptor gamma coactivator 1 α), is encoded by a nuclear gene. The fact that the mitochondrion retains its own genome poses logistic problems for the regulation of mitochondrial physiology because the relevant transcriptional programs occur in two distinct cellular compartments. The phenomenon of retrograde signaling (in which signals originating from the mitochondrion are transmitted to nuclear transcriptional responses) provides a level of coordination between the two transcriptional programs, and the protein products of nuclear-encoded genes can be imported into the mitochondrion. Furthermore, nuclear transcription factors such as STAT3 and the androgen receptor (AR) have been shown to be imported into the mitochondria to regulate mitochondrial genes (7, 8). This study adds the prostate differentiation factor NKX3.1 to this list and attributes a role for it in driving expression of components of the respiratory chain. The AR, like NKX3.1, has a key role in prostate homeostasis and disease. Indeed, NKX3.1-expressing progenitor cells, castration-resistant NKX3.1 cells (CARNS), promote prostate regeneration after androgen deprivation (4). Thus, it would be interesting to understand whether the nuclear-mitochondrial communication is regulated diversely by NKX3.1 and AR in prostate stem/progenitor and differentiated cells in prostate homeostasis and tumorigenesis. Overall, future studies will provide understanding of how these dual functions are coordinated, and whether a dysfunctional regulation of the nuclear-mitochondrial axis may not only contribute to tumor initiation, but also seed novel therapeutic opportunities for patients to prevent aggressive disease.

Paraquat promotes leakage of electrons from electron transport chains, and it is therefore a powerful inducer of mitochondrial ROS in mammalian cells. Its use in this study enabled the determination of a novel mechanism of regulation of mitochondrial homeostasis. However, few

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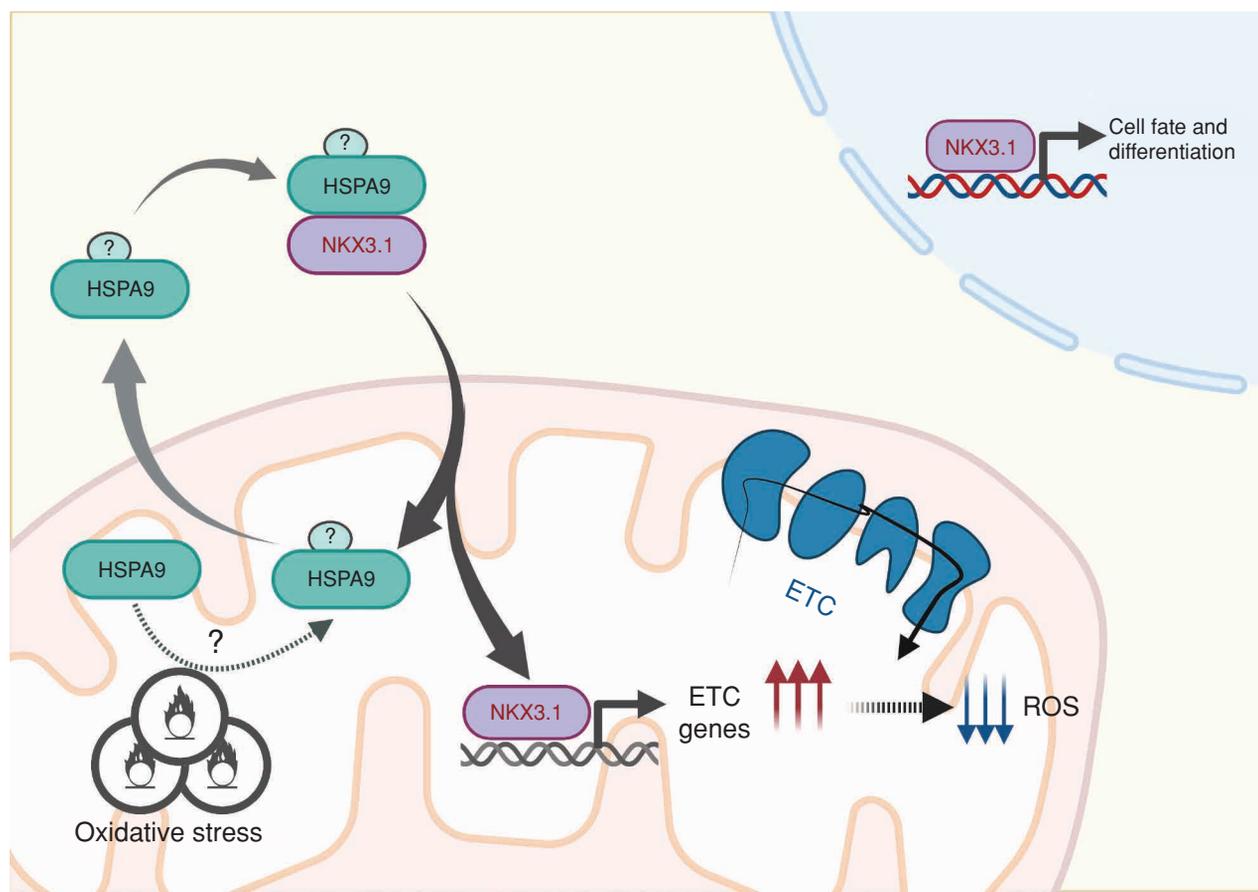


Figure 1. The NKX3.1 homeobox transcription factor is transported into mitochondria by the chaperone HSPA9 upon oxidative stress, while the nuclear pool is maintained. The mitochondrial localization of NKX3.1 results in the transcriptional activation of components of the mitochondrial electron transport chain and, therefore, a decrease of ROS. It is still unknown whether posttranslational modifications may play a role in HSPA9 export from the mitochondria or whether there are modifications required for the interaction with cytoplasmic NKX3.1. ETC, electron transport chain; ROS, reactive oxygen species.

mitochondria encounter ROS on this scale under physiologic, or indeed pathophysiologic, conditions. It seems likely that under more subtle conditions of mitochondrial ROS, the same pathway should be activated, albeit in more subtle fashion. It will therefore be important to determine the extent to which mitochondrial NKX3.1 activity responds to more subtle perturbations of mitochondrial homeostasis and whether this axis can be a therapeutic target in pathologic states.

In mechanistic terms, this study indicates that there must be a signal that initiates the HSPA9-mediated recruitment of NKX3.1 for its consequent mitochondrial import. Because the primary site of paraquat action is in the mitochondrion in mammalian cells, one would expect this signal to originate there (Fig. 1). Such a signal could include post-translational modification (PTM) of either protein (9, 10) or, perhaps, association with another molecule and proteomic approaches may help to identify this aspect of the pathway. Similarly, such a modification or interaction would normally be reversible, and so there may be enzymatic removal of a PTM when homeostasis is restored. Finally, it may emerge that other nuclear factors are similarly recruited and imported to the mitochondrion by HSPA9.

Although the authors demonstrate that the mitochondrial NKX3.1 is not transported from the nucleus, they do not touch upon whether the mitochondrial NKX3.1 protein requires increased transcription, and whether RNA stability is extended under oxidative stress, to provide extra protein for mitochondrial localization. As current mouse and preclinical models, including germline deletion of *NKX3.1* (*Nkx3.1^{-/-}*), impair both nuclear and non-nuclear functions of these homeobox transcription factors, it may prove tricky to fully separate the nuclear and mitochondrial functions of NKX3.1. The authors take an elegant approach to this question through the use of point mutants that perturb specific functions, and other novel models modulating the nuclear and mitochondrial functions may contribute to better understanding of the cooperation between the nucleus and the mitochondrial functions, particularly under stress conditions.

The authors show that the NKX3.1-mediated induction of mitochondrial genes encoding components of the respiratory chain protects against ROS, and this poses a conundrum. Because the protein components of the respiratory chain are mainly encoded by the nuclear genome, why does induction of only the mitochondrial subset protect against ROS? One

would imagine that coordinated induction of both nuclear and mitochondrial respiratory genes would be required for a stoichiometrically balanced homeostatic response. In turn, this poses a question of evolutionary importance: is there a selective pressure for the mitochondrion to retain the specific respiratory genes in its genome (e.g., for ROS protection) or has the loss and retention been purely random? Such a question is hard to address experimentally but is fascinating nonetheless.

Finally, the implications of this study for prostate cancer are that mitochondrial ROS are drivers of this disease and that resolution of mitochondrial ROS may be protective. The use of antioxidants in patients may be a double-edged sword, because their use can lead to detrimental outcomes in some tumor types. However, individuals harboring NKX3.1 polymorphisms that lead to elevated risk of prostate cancer may benefit from prophylactic antioxidant therapy, particularly for mitochondrial antioxidants.

Authors' Disclosures

No disclosures were reported.

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