ANTIOXIDANTS & REDOX SIGNALING
Volume 14, Number 4, 2011

DOI: 10.1089/ars.2010.3778

FORUM EDITORIAL

Forkhead Box O Transcription Factors: Key Players in Redox Signaling

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Abstract

The Forkhead Box O (FOXO) transcription factors are famous for their role in longevity: both *Caenorhabditis elegans* and *Drosophila melanogaster* can extend their median and maximum lifespan in a FOXO-dependent manner, and certain single nucleotide polymorphisms in human FOXO genes are associated with reaching an age above 100 years. Ablation of FOXO1, 3a, and 4 in adult mice predisposes them to tumorigenesis and stem cell depletion, and the latter could at least partly be reversed by treatment with antioxidants. Indeed, FOXO has been known to regulate the defense against reactive oxygen species through transactivation of antioxidant genes like manganese superoxide dismutase and catalase. At the same time, reactive oxygen species regulate FOXO activity in many ways through an elaborate combination of activating as well as repressing post-translational modifications, including phosphorylation, acetylation, ubiquitinylation, and methylation. Hence, FOXO is at the centre of redox signaling, but it is unclear whether and how exactly redox signaling to and from FOXO contributes to its effects on longevity. In this forum issue we give an overview of the many facets of FOXO in worms, flies, mice, and humans. *Antioxid. Redox Signal.* 14, 559–561.

Although a recent study showed that the induction of autophagy through Forkhead Box O (FOXO) can occur independent of its transcriptional activity (16), the effects of FOXO on cellular and organismal physiology are predominantly exerted through their transcriptional activation (or repression) of a large number of target genes. Van der Vos and Coffer have reviewed the known transcriptional targets of the FOXO family members (14). They have described the functional consequences of activation of these targets and sorted them according to the cellular process they are involved in. The DNA-binding domain of the FOXO family is highly conserved both across family members and across species. This would suggest that there is redundancy in function for the different FOXO family members. Nevertheless, single knockout mice of FOXO1, FOXO3, and FOXO4 also have different phenotypes, suggesting that there are specialized roles for the different FOXO family members. Van der Vos and Coffer briefly discuss the occurrence of redundant *versus* overlapping functions and suggest that differential transcriptional activities of the FOXO family members may derive not only from differences in tissue distribution, but also from differential association with transcriptional cofactors that might again be cell type specific (14). The observed redundant *versus* overlapping functions of FOXO family members in the immune system are further elaborated on in the review by Dejean *et al.* (4) (see also below).

FOXO controls the turnover of reactive oxygen species (ROS) through the transcriptional regulation of a set of antioxidant enzymes, including manganese superoxide dismutase, catalase, and peroxiredoxins. On the other hand, the activity of FOXO itself is altered when it becomes for instance phosphorylated by Jun N-terminal kinase (JNK) and acetylated by CBP/p300 when ROS is encountered. An original research forum communication by Szypowska *et al.* in this issue adds another ROS-mediated regulatory pathway that controls FOXO activity: the authors show that FOXO4 becomes inhibited through phosphorylation by Nemo-like kinase upon ROS treatment (11). Hence, both inhibitory and activating post-translational modifications occur on FOXO upon ROS treatment. This may reflect a fine-tuning mechanism that allows FOXO to be controlled differentially in response to low or high cellular ROS levels. The scavenging of ROS through activation of FOXO and the regulation of FOXO activity by ROS indicates the interconnection of FOXO and redox signaling. The forum review by Storz outlines how FOXO and redox signaling regulate each other and how this contributes to the various physiological roles of FOXO (10). Storz argues that the ROS scavenging properties induced by FOXO transcriptional targets may be beneficial in terms of tumor suppression and longevity as long as a cell has not undergone oncogenic transformation, but that the same ROS scavenging properties might actually help tumor

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cells survive starvation or drug treatment. In line with this, a recent article showed that indeed antioxidants could help tumor cells survive oxidative stress that derives from metabolic defects caused by loss of matrix attachment that precedes invasion (9).

As outlined in the review by Storz, FOXO activity can be controlled by ROS, but the classical mode of FOXO regulation is through the insulin signaling pathway. Insulin signaling activates PKB/Akt downstream of PI3Kinase. PKB/Akt phosphorylates FOXO, which leads to binding of 14-3-3 and subsequent nuclear export and thus transcriptional inactivation of FOXO. Hence, FOXO is both positively regulated by JNK-mediated phosphorylation and negatively regulated by PKB-mediated phosphorylation. On top of this, a number of other post-translational modifications control the activity of FOXO. Van den Berg and Burgering discuss how these opposing signals are integrated to result in a particular FOXO-dependent response (13).

Much of the seminal work on elucidation of the many functions of FOXO transcription factors has been done or been put in perspective using animal model systems. The key roles of daf-16 (the worm FOXO homolog) in stress resistance and longevity, for instance, was originally discovered in the worm *Caenorhabditis elegans*. Yen *et al*. have reviewed the various aspects of daf-16 (15). Like worms, flies have a single FOXO gene, dfoxo, and Puig and Mattila have summarized the current knowledge of dFOXO in the fruit fly *Drosophila melanogaster* (8). The mechanisms found to control regulation of FOXO in worms and flies are largely conserved in mice and human cells, illustrating that these relatively simple but very powerful genetic model systems are of great value in studying FOXO in humans.

The best studied FOXO isoform in mice is FOXO1, and both loss-of-function and gain-of-function FOXO1 models in various tissues have been generated over the years. FOXO1 has been shown to play important roles in the regulation of metabolism, which are reviewed by Cheng and White (2). They describe how FOXO1 could be a future therapeutic target for metabolic diseases with an insulin resistance component like obesity and diabetes. The FOXO transcription factors are also important in the cells of the immune system, and the review by Dejean *et al*. discusses the implications of several studies using immune-system-specific FOXO mouse models (4). They further go into specialized and redundant functions of the different FOXO isoforms in the immune system and how specialized functions may arise despite the fact that all FOXO isoforms recognize the same DNA-binding domain. As noted in the latter review, an important step forward in understanding overlapping and distinct roles of FOXO isoforms was the joined efforts of the DePinho and Gilliland labs to construct and analyze conditional FOXO1, FOXO3a, and FOXO4 knockout mice. Two important overlapping functions for FOXO1, FOXO3a, and FOXO4 isoforms were described in the first two articles that described these conditional mouse models: hematopoietic stem cell maintenance and tumor suppression (7, 12). FOXOs had already been predicted to be tumor suppressors [for a review, see Dansen and Burgering (3)], but this was the first study to show that this was indeed the case at endogenous levels. The final review in this forum issue by Myatt *et al*. describes the various aspects of FOXO as a tumor suppressor and as a potential target in cancer treatment (6). They implicate redox signaling upstream of FOXO as a major player in the desired cellular responses to chemotherapy: arrest and apoptosis. On the other hand, they describe how the upregulation of the ROS scavenging system by FOXO also plays a role in the development of resistance to anti-cancer treatment.

Taken together, I think that this forum issue gives a nice overview of the many aspects of FOXO transcription factors ranging from detailed molecular mechanisms of regulation to their use as potential therapeutic targets. The FOXO field is a good example of how important it is that researchers from different backgrounds and working with different model systems look across the borders to come to new insights, and that work on tissue culture systems or simple genetic organisms can prove to be of importance in understanding human disease. I think that it is clear from this forum issue that FOXO transcription factors are key players both in redox signaling and in longevity. However, it is less clear how exactly FOXO-dependent redox signaling and longevity are linked. The idea that merely the upregulation of the antioxidant status is what controls longevity is most certainly too simple, and there actually is evidence that this is indeed not the case (1, 5). The key might be in fine-tuning FOXO activity in response to certain ROS levels to lead to the most appropriate cellular decision (arrest and repair or initiate apoptosis or senescence) in terms of longevity.

Acknowledgments

The author would like to thank all the authors of the forum reviews on FOXO for their contributions to this forum issue and my colleagues and others for helping out peer reviewing all these forum reviews. Tobias B. Dansen is being sponsored by a grant from the Dutch Cancer Society/KWF Kankerbestrijding.

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Date of first submission to ARS Central, November 13, 2010; date of acceptance, November 13, 2010.

Abbreviations Used

FOXO = Forkhead Box O
PKB = protein kinase B
ROS = reactive oxygen species

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