FOXO Transcription Factors: Regulators of Metabolism and Stress Resistance [†]

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Abstract: FOXO (Forkhead box, class O) proteins are transcriptional regulators ubiquitously expressed in mammalian cells with roles in modulating fuel metabolism, stress resistance and cell death. FOXO transcription factors are regulated by redox processes at several levels, including enzymatic and nonenzymatic posttranslational modification. Target genes controlled by FOXO proteins include genes encoding antioxidant proteins, thus likely contributing to the key role FOXOs play in the cellular response to oxidative stress. Here, an overview is provided on (i) the modulation of FOXO proteins by thiol depleting agents, (ii) consequences of thiol depletion for stress resistance and life span of a model organism, *Caenorhabditis elegans* and (iii) the role of FOXO proteins therein.

Keywords: hormesis; diethyl maleate; glutathione; *C. elegans*; stress signaling; Nrf2

1. Introduction

FOXO transcription factors are regulated by posttranslational modification, including phosphorylation, acetylation and ubiquitinylation. In addition, nonenzymatic modification, such as thiol oxidation to generate intermolecular disulfide bonds between FOXOs and regulating proteins was described to occur under exposure to reactive oxygen species, such as hydrogen peroxide [1], constituting one of the several layers of redox regulation of these transcription factors [2].

Here, we summarize recent data on the consequences of cellular thiol depletion for FOXO activity and organismal life span in addition to reviewing the role of individual cysteine residues in controlling their transcriptional regulation by FOXOs.

2. Hormetic Effects of Thiol Depletion in *Caenorhabditis elegans*

Exposure of a model organism, *C. elegans*, to thiol-depleting agents, such as the alkylating agent diethyl maleate (DEM), causes a moderate depletion of glutathione and a modulation of the organism's life span. Whereas growth of worms on agar containing 1 mM DEM significantly shortens life span by approx. 10% relative to control conditions, an exposure of worms to lower concentrations of DEM (up to 0.1 mM) elicits an extension of life span (by approx. 5%), pointing to an adaptive response elicited by lower DEM concentrations [3]. In fact, resistance to oxidative stress elicited by the redox cyler paraquat is also elevated in worms exposed to lower DEM concentrations. Interestingly, the same low DEM concentrations stimulate the expression of genes encoding antioxidative proteins. Additionally, Thiol depletion elicited by attenuation of glutathione biosynthesis through RNA interference with expression of *C. elegans* γ -glutamyl-cysteine synthetase confirms that glutathione depletion may cause an adaptive response eliciting life span extension [3].

DEM-induced life span extension is mediated, in part, by the *C. elegans* FOXO ortholog, DAF-16, as demonstrated by RNA interference. In addition to DAF-16, the *C. elegans* ortholog of transcription

factor Nrf2, SKN-1, appears to be involved in the adaptive response, in line with the known Nrf2 activating activity of DEM [4].

3. Diethyl Maleate in Mammalian Cells: Glutathione Depletion and FOXO Modulation

Although DEM affects *C. elegans* life span via modulation of the nematode FOXO ortholog, no change in transcriptional regulatory activity is observed for mammalian FOXO1 in cells exposed to DEM [5]. Despite a nuclear accumulation of FOXO1 that occurs in response to exposure of human HepG2 hepatoma cells to DEM, no upregulation of FOXO1 target genes is observed—quite in contrast to Nrf2 target genes, which are upregulated at the same time. Moreover, FOXO1 remains located in the nucleus even in the presence of insulin, a stimulus eliciting FOXO inactivation and nuclear exclusion. In summary, DEM traps inactive FOXO1 in the nucleus whereas it stimulates Nrf2-dependent gene expression [5].

4. Distinct Roles of FOXO1 Cysteine Residues in Modulating Transcriptional Activity

Analysis of the contributions of individual cysteine residues of human FOXO1 to its transcriptional regulatory activity through site-directed mutagenesis indicates that only one of the seven cysteines is required for full basal activity [6]. Cys-612, even under basal culture conditions, mediates the stimulatory effects of transcriptional coregulators, such as CBP or PGC1 α . Interestingly, the extent of Cys-612 contribution is dependent on the promoter context, as demonstrated with two different FOXO1 target promoters, those of the genes coding for glucose 6-phosphatase (catalytic subunit) and of selenoprotein P [6].

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