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The role of peroxiredoxin 4 in inflammatory response and aging

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ABSTRACT

In prior studies, we determined that the moderate overexpression of the *Drosophila* endoplasmic reticulum (ER)-localized peroxiredoxin (Prx), dPrx4, reduced oxidative damage and conferred beneficial effects on life span, while a high-level expression increased the incidence of tissue-specific apoptosis and dramatically shortened longevity. The detrimental pro-apoptotic and life-shortening effects were attributed to aberrant localization of dPrx4 and the apparent ER stress elicited by dPrx4 overexpression. In addition, the activation of both the NF-κB- and the JAK/STAT-mediated stress responses was detected, although it was not clear whether these served as functional alarm signals.

Here we extend these findings to show that the activation of the NF-κB-dependent immunity-related/inflammatory genes, associated with life span shortening effects, is dependent on the activity of a *Drosophila* NF-κB ortholog, Relish. In the absence of Relish, the pro-inflammatory effects typically elicited by dPrx4 overexpression were not detected. The absence of Relish not only prevented the hyperactivation of the immunity-related genes but also significantly rescued the severe shortening of life span normally observed in dPrx4 overexpressors. The overactivation of the immune/inflammatory responses was also lessened by JAK/STAT signaling. In addition, we found that cellular immune/pro-inflammatory responses provoked by the oxidant paraquat but not bacteria are mediated via dPrx4 activity in the ER, as the upregulation of the immune-related genes was eliminated in flies underexpressing dPrx4, whereas immune responses triggered by bacteria were unaffected. Finally, efforts to reveal critical tissues where dPrx4 modulates longevity showed that broad targeting of dPrx4 to neuronal tissue had strong beneficial effects, while targeting expression to the fat body had deleterious effects.

1. Introduction

Changes in redox-sensitive signaling due to fluctuations in the cellular redox milieu lead to functional consequences in a variety of biological processes, including stress response, inflammation, and apoptosis. While such responses are potentially adaptive in nature, permitting cells to overcome various exogenous and endogenous challenges, chronic, or inadequate responses may have a deleterious impact. Consequently, a robust redox homeostatic response is considered essential in maintaining healthy cell function, whereas its disruption may inform/promote a multitude of disease states.

A pivotal role in maintaining cellular redox homeostasis and proper redox-sensitive signaling belongs to the peroxiredoxins, a family of thiol-dependent peroxidases that possess the capacity to act as redox sensors. Peroxiredoxins (Prx) not only have the capacity to control local hydrogen peroxide fluxes but have also been shown to transmit H_2O_2-mediated protein sulfhydryl modifications to specific target proteins [1]. Endoplasmic reticulum (ER) is one of the major sources of cellular H_2O_2, where it is produced during the oxidative disulfide formation of newly synthesized proteins [2]. Recently, we reported that Prx4, which resides in the ER, is involved in modulating *Drosophila* longevity under normal conditions or in the presence of various environmental stressors [3]. Prx4 serves a dual function in the ER. It both maintains proper H_2O_2 concentrations, generated during protein oxidative folding [4], and also uses hydrogen peroxide to catalyze the formation of disulfide bonds in protein disulfide isomerase [5,6]. The moderate ectopic overexpression of Prx4 was found to have beneficial effects on fly physiology, including a significant extension in life span. By contrast, the global high-level overexpression of dPrx4 (>5-fold) elicited increased incidence of tissue-specific apoptosis and shortened life span concomitant with overproduction of antimicrobial peptides (AMP), a signature of the NF-κB signaling in the immune and inflammatory responses [3], and is reminiscent of the alarm signal triggered by ER stress due to accumulation of unfolded proteins, the so-called unfolded protein response (UPR) [7,8]. The activation of the NF-κB signaling pathway is presumed to safeguard cellular viability and function, but when chronically induced, it may become disruptive, leading to cell suicide as a last resort to dispose of dysfunctional cells where the accumulation of misfolded proteins has overwhelmed repair capacity [9].

Abbreviations: ER, endoplasmic reticulum; Prx, peroxiredoxin; AMP, antimicrobial peptide; UPR, unfolded protein response; Act, actin; Da, daughterless; AttD, attacin D; Dipt, diptericin; Drs, drosomycin; TotA, Turandot A; ROS, reactive oxygen species.

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The modulation of the ER redox domain by manipulating Prx4 levels provides the opportunity to experimentally establish a state of chronic inflammation and identify specific pathway elements that mediate this ER-derived stress and thus facilitate the design of interventions to manage inflammatory responses and apoptosis.

2. Materials and methods

2.1. Fly strains and procedures

The UAS-dPrx4 transgenic lines, RNAi-*dPrx4* mutants, RNAi mutants for the genes representing the JAK/STAT pathway (*hopscotch*, *Stat92E*, and *domeless*), and driver fly lines were those described by Radyuk et al. [3]. The S106-inducible GeneSwitch-GAL4 fat body-specific driver was kindly supplied by Blanka Rogina (University of Connecticut Health Science Center). A null mutant strain for the NF-κB-like transcription factor Relish, rel^{E20}, was obtained from the Bloomington Stock Center. All fly lines were backcrossed into our reference *y w* genetic background.

Fly collection and husbandry were as described by Radyuk et al. [3]. Briefly, flies overexpressing dPrx4 at high levels and flies overexpressing dPrx4 in combination with *domeless* RNAi-mediated underexpression were developed at 18 °C because of the lethal effects on development at 25 °C. Adults were reared at 25 °C after hatching. The underexpression of Hopscotch and Stat92E by RNAi was achieved with high-level global *daughterless* (Da) or Actin (Act) GAL4 drivers, and the overexpression of dPrx4 with S106-Switch driver was achieved by feeding flies food supplemented with 100 μg/ml mifepristone (RU486). Controls included the RNAi transgene target alone and the driver alone except for the S106 GeneSwitch experiment where genetically identical flies fed food containing the mifepristone solvent, ethanol, served as control.

Responses to oxidants and infection were studied by feeding flies with 1% sucrose solution containing 10 mM paraquat or septically injuring flies by pricking with a needle dipped in an *Escherichia coli* suspension, as detailed by Radyuk et al. [3]. Survivorship studies were conducted as described previously [10].

2.2. RT-PCR

Primers specific for AMP genes and Turandot A (TotA) were those indicated in publications [3,11]. Real-time PCR analysis was performed as described previously [11], where PCR reactions were performed with SYBR Green fluorescent dye (Molecular Probes), and the signals obtained for each gene were standardized against signals obtained for the *rp49* housekeeping gene.

2.3. TUNEL labeling

The assessment of apoptosis-induced DNA fragmentation was made in cryosections prepared from whole flies using the In Situ Cell Death Detection Kit, TMR red (Roche, Indianapolis, IN, USA), as detailed previously [3]. Images were acquired by fluorescence microscopy (Zeiss), using AxioVisionLE4_3 software.

2.4. Statistical analysis

All statistics were calculated using Prism for Macintosh version 4.0a software (GraphPad Software, Inc.). Differences in mRNA levels were compared between groups by analysis of variance. In studies of life span, the mean survivorship time and differences between survivorship curves were assessed using the log-rank test.

3. Results

3.1. Induction of AMPs and TotA in response to Prx4 overexpression requires Relish

As reported by us previously [3], dPrx4 overexpression at high levels (>5-fold) caused an increase in the expression of both the NF-κB-dependent immune response/pro-inflammatory genes, AMPs, and the cytokine-like protein Tot A, a target of JAK/STAT signaling. Given that the NF-κB transcription factor Relish has been identified as a common regulator of the expression of AMPs and Turandot proteins [12] as well as a mediator of responses elicited due to ER stress [8], we postulated that the stress response due to Prx4 overload is mediated via Relish. To test this idea, we measured the Prx4-driven stress response in the absence of Relish using the rel^{20} null mutant.

The results of this analysis, as shown in Fig. 1, revealed that the activity of Relish is indeed required for induction of AMPs and TotA in response to the apparent ER stress caused by dPrx4 overload. The upregulation of the AMPs attacin D (AttD) and diptericin (Dipt), as well as TotA, normally observed in flies overexpressing dPrx4, was completely eliminated in the *relish* mutant. We also observed lower levels of drosomycin (Drs) in flies underexpressing Relish. Notably the basal levels of AMPs were lower in 10 days old rel^{20} mutant flies compared to controls. It is tempting to conjecture that this transcription factor is also critical for the age-dependent increase in AMP transcripts levels that occurs in normal flies.

3.2. JAK/STAT signaling mitigates the activation of NF-κB-dependent immunity genes

JAK/STAT signaling has been previously shown to repress NF-κB-dependent immune signaling. In a series of SL2 cell culture studies, RNAi was used to reduce levels of the JAK/STAT signaling factor Stat92E, giving rise to a significant increase in the expression of Relish-mediated immunity genes and providing support for the notion that Stat92E serves as a Relish antagonist [13].

To determine whether a similar relationship could be discerned in response to stress elicited by dPrx4 overexpression, we analyzed the expression of AMP genes in RNAi mutant flies underexpressing three distinct elements of the JAK/STAT pathway (Stat92E, Hopscotch, and Domeless) in the presence or absence of Prx4 overexpression. Consistent with previous findings ((3) and Fig. 1), the overexpression of dPrx4 alone at high levels resulted in AMP induction, particularly evident for AttD and Dipt (Fig. 2). The underexpression of the *domeless* and *Stat92E* genes of the JAK/STAT pathway resulted in increases in AMPs similar to those observed for Prx4 overexpression alone, while the underexpression of *hopscotch*, the *Drosophila* JAK homologue, resulted in a particularly strong increase in AMP levels relative to those elicited by Prx4 overexpression and comparable to the increase during aging (55-day-old control). The AMP activation effects were not diminished in response to a combination treatment (Prx4 overexpression + JAK/STAT knockdown), and in fact some synergy was evident in three cases (See arrows, Fig. 2). Overall, these results support the notion that JAK/STAT signaling plays an inhibitory role in the Relish-dependent activation of AMPs.

3.3. dPrx4 is required for induction of Relish-dependent AMPs in response to PQ but not infection

There is evidence that exposure to oxidants or infection (particularly viral) may provoke ER stress and, as a consequence, lead to pro-inflammatory responses mediated via the NF-κB pathways [8,9]. We reasoned that if the ER-specific Prx4 mediates AMP activation in response to oxidative stress and septic injury, the knockdown of Prx4 would abolish this activation effect. Consequently, levels of Prx4 were knocked down >90% by RNAi, and flies were subsequently subjected