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Aging attenuates redox adaptive homeostasis and proteostasis in female mice exposed to traffic-derived nanoparticles ('vehicular smog')

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ABSTRACT

Environmental toxicants are catalysts for protein damage, aggregation, and the aging process. Fortunately, evolution selected adaptive homeostasis as a system to mitigate such damage by expanding the normal capacity to cope with toxic stresses. Little is known about the subcellular degradative responses to proteins oxidatively damaged by air pollution. To better understand the impact of environmental toxicants upon the adaptive homeostatic response, female C57BL/6 mice were exposed for 10 weeks to filtered air or reaerosolized vehicular-derived nano-scale particulate matter (nPM), at which point tissues from young (6 month) and middle-aged (21 month) mice were studied. We found significant increases of proteolytic capacity in lung, liver, and heart. Up to two-fold increases were seen in the 20S Proteasome, the Immunoproteasome, the mitochondrial Lon protease, and NF-E2-related factor 2 (Nrf2), a major transcriptional factor for these and other stress-responsive genes. The responses were equivalent in all organs, despite the indirect input of inhaled particles to heart and liver which are downstream of lung. To our knowledge, this is the first exploration of proteostatic responses to oxidative damage by air pollution. Although, middle-aged mice had higher basal levels, their Nrf2-responsive-genes exhibited no response to nanoparticulate exposure. We also found a parallel age-associated rise in the Nrf2 transcriptional inhibitors, Bach1 and c-Myc which appear to attenuate adaptive responses in older mammals, possibly explaining the 'age-ceiling effect.' This report extends prior findings in male mice by demonstrating the involvement of proteolytic responses to traffic-related air pollution in lung, liver, and heart of female mice, with an age-dependent loss of adaptive homeostasis.

1. Introduction

Chronic exposure to traffic-related air pollution (TRAP) particulate matter can have deleterious health consequences, evident in high-smog environments. Even more troubling is the impact of daily low level exposure to a subset of TRAP-derived particles ($PM_{2.5}$) [1] upon human health. In the Women's Health Initiative Memory Study (WHIMS) cohort of 65,000 postmenopausal women, chronic exposure to air

Abbreviations: nPM / $PM_{0.2}$, Nano-Scale Particulate Matter / Particulate Matter < 0.2 μm diameter; $PM_{2.5}$, Particulate Matter < 2.5 μm diameter; Nrf2, Nuclear factor (erythroid-derived 2)-like 2; Bach1, BTB Domain And CNC Homolog 1; c-Myc, MYC proto-oncogene; TRAP, Traffic-related air pollution; WHIMS, Women's Health Initiative Memory Study; Keap1, Kelch Like ECH Associated Protein 1; ARE, Antioxidant Response Element; EpRE, Electrophile Response Element; HO-1, Heme Oxygenase-1; NQO1, NAD(P)H Quinone Dehydrogenase 1; SOD1, Superoxide Dismutase 1; *C. elegans*, Caenorhabditis elegans; *D. melanogaster*, Drosophila melanogaster; [^{3}H]Hb, Tritium-tagged hemoglobin; [^{3}H]$Hb_{oxdized}$, Tritium-tagged oxidized-hemoglobin; ATP, Adenosine Triphosphate; LMP2, Proteasome Subunit Beta-1i; LMP7, Proteasome Subunit Beta-5i; GCLC, Glutamate-Cysteine Ligase Catalytic Subunit; GCLM, Glutamate-Cysteine Ligase Modifier Subunit; CSC, Cigarette smoke condensate; BCA, Bicinchoninic acid; Z-LLG-AMC, Z-Leu-Leu-Glu-AMC; Z-ARR-AMC, Z-Ala-Arg-Arg-AMC; Suc-LLVY-AMC, Suc-Leu-Leu-Val-Tyr-AMC; Ac-PAL-AMC, Ac-Pro-Ala-Leu-AMC; Ac-ANW-AMC, Ac-Ala-Asn-Trp-AMC; AMC, 7-amino-4-methylcoumarin; H_2O_2, Hydrogen peroxide; BSA, Bovine Serum Albumin

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pollution, consisting of $PM_{2.5}$, which is above EPA standards, increased the risk of cardiovascular disease [2]. Similarly, exposure to TRAP-derived particles promotes inflammation in multiple tissues [3,4], including the small airways, further exacerbating asthma and chronic bronchitis development [5]. Moreover, a WHIMS subset had nearly 2-fold higher dementia from excess $PM_{2.5}$ exposure [6]. Additionally, chronic exposure to TRAP particulate matter leads to cumulative and harmful changes in healthy older adults, resulting in increased mortality [7]. Yet, our mechanistic understanding behind TRAP-promoted morbidity and mortality is limited. Due to the cellular stress response being crucial for cellular homeostasis, it is paramount we understand how this pathway is impacted following particulate exposure. Here, we address the interaction between aging and a relatively unexplored subfraction of ambient $PM_{2.5}$, termed nanoscale particulate matter (nPM, $PM_{<0.2}$), which lacks black carbon and water-insoluble organics, with particle diameter of 0.2 μm and smaller (nPM, $PM_{<0.2}$) [8]. Prior work has identified this subset of $PM_{2.5}$-derived particles as having greater cytotoxicity [9] and to act as a pro-inflammatory inducer [8] compared to larger PM.

Cellular stress response pathways are evolutionarily-conserved mechanisms that attenuate and remove cellular damage to lipids, proteins, and DNA [10]. Dysregulated protein homeostasis and adaptive stress responses are considered 2 of the 7 contributing factors of aging [11]. A major enzyme of the proteostasis pathway is the 20S proteasome and is the primary means for protein turnover within the cell [12]. It consists of four rings comprised of 2-outer alpha rings, necessary for substrate-recognition, and two inner beta rings, necessary for catalytic activity: caspase-like (β_1), trypsin-like (β_2), and chymotrypsin-like (β_5) activity [12]. Upon activation of the adaptive stress-protective response [13], an immediate pool of 20S proteasome becomes available (~ 1 h), due to HSP70-mediated sequestering of the highly oxidant-inactivated 19S regulatory caps and subsequent dissociation of the 26S proteasome [14,15]. Concurrently, Nrf2-mediated transcriptional up-regulation of de-novo 20S proteasome subunits, further increases the available pool of 20S proteasome (within 16 h) [16]. Thus the homeostatic role of Nrf2-activation of the 20S proteasome is crucial for protein quality control.

A key lynchpin of the adaptive stress-protective pathway is NF-E2-related factor 2 (Nrf2), a crucial master transcriptional regulator of many Phase II detoxification and stress responsive enzymes. Nuclear translocation of Nrf2 is induced by diverse stressors, including exogenous oxidants [16,17], heavy metals [18], and nPM exposure [19]. Additionally, ultrafine particle exposure (< 0.2 μm) increased Nrf2-targeted stress responsive genes [20]. Under non-stressed conditions, Nrf2 is continuously synthesized and degraded by a mechanism dependent upon its interaction with Keap1, which facilitates its ubiquitination. The ubiquitinated Nrf2 is then degraded by the 26S Proteasome [21]. This limits Nrf2 translocation into the nucleus. Electrophilic modifications of Keap1, which occurs continuously, but is increased most during oxidative stress, prevents its binding to Nrf2. In turn, enabling newly synthesized Nrf2 to escape degradation and to promote its nuclear accumulation. Once in the nucleus, Nrf2 binds to electrophile response elements (EpRE, which are also known by the misnomer, antioxidant response elements or ARE) in the promoter regions of Phase II genes (such as HO-1 [22], GstDs [23], NQO1 [24] and stress-responsive proteins, including the 20S Proteasome [25] and SOD1 [26]).

During aging, basal Nrf2 activation is highly tissue-dependent. Moreover, there is high age-dependent variability in Nrf2 levels pervasive in the literature. Some studies suggest Nrf2 levels decrease in rat liver from 20 month old animals [27,28], whereas, other studies report Nrf2 levels are suggested to rise in aged tissue [29]. Indeed, Nrf2 variability in aging is evident when assessing the age-associated impact upon the unstressed levels of Phase II detoxification genes, which are inconsistent in regards to direction and the extent of change with age [30–32].

Consequently, the 20S proteasome is negatively impacted by the dysregulation of Nrf2 transcriptional activity. Numerous studies have assessed the age-associated impact upon 20S expression and activity, but without conclusive findings. Variation arises between tissues [33–36], species [37,38], and the sexes [39–41]. In contrast, one age-dependent trend remains consistent: the age-related inability to activate Nrf2-dependent stress-responsive genes [24]. In other words, the basal expression of Nrf2-dependent genes varies markedly in aging, but the ability to respond to oxidative challenges consistently declines with age.

Prior work has shown that mild and transient exposure to low doses of an oxidant (such as hydrogen peroxide) is capable of activating the Nrf2-mediated pathway without causing undo cellular harm [42,43]: a process dubbed ‘adaptive homeostasis’ [10]. This process is demonstrated in cell culture [44] and the model organisms *C. elegans* [45] and *D. melanogaster* [39,46–48]. In order to better understand the conserved role of the adaptive homeostatic response, requires understanding if similar changes arise in a mammalian system. One such physiologically relevant approach is to expose mice (at different ages) to sub-lethal amounts of traffic-derived nanoparticulate matter (nPM) [19]. In turn, improving our understanding of the adaptive homeostatic stress response, especially in the context of environmental toxicants.

A highly relevant aspect of the present study is the exploration of sex-dependent differences in a mammalian model. Earlier studies have demonstrated a clear sexual dimorphism in stress protective responses and activation of the adaptive response in lower organisms [49,50]. Moreover, sex is a strong predictor of lifespan in mammals, with females typically outliving males [51,52]. However, actually testing whether sex differences in the regulation of the adaptive response is conserved in a mammalian model has been limited. The present study addresses the exploration of the female-specific response to short-term, sub-lethal amounts of traffic-derived nanoporticulate matter. Although our study is not a direct, side-by-side comparison of males and females, our findings do offer an important comparison of females studied under identical experimental protocols as those used in our previous report of male adaptive responses [19]. The present work also explores the consequences of sub-lethal exposure to environmental particles (‘smog’), which has been previously implicated in accelerating neurological pathologies [53,54], including Alzheimer's disease [55] which is a highly female-favored disease [6], associated with the loss of proteostasis and Nrf2 regulation [56,57].

The majority of the work associated with our understanding of the adaptive homeostatic stress response has centered upon changes related to Nrf2. Yet, other regulators, which directly interact with Nrf2, are worth closer examination. One such is c-Myc, primarily known for its role as a proto-oncogene [58], with more recent findings indicating its interaction with Nrf2 [59]. Specifically, c-Myc silencing leads to the prolonged half-life of nuclear Nrf2, in both basal and induced conditions [59], suggesting c-Myc may play a role in nuclear Nrf2 turnover. The constitutively expressed Bach1 acts as an Nrf2 nuclear antagonist, which also binds to EpRE and thereby competes with Nrf2-mediated gene expression [60,61]. However, during oxidative stress, Nrf2 is imported into the nucleus, while Bach1 dissociates from EpREs. This suggests that Bach1's role as a suppressor may be a necessary means of dampening the Nrf2 response, and potentially serving as an ‘off-switch’ for the adaptive homeostatic response.

Lastly, it is notable that the majority of prior work on aging relies upon comparing a young organism to an aged organism. Though relevant in furthering our understanding about the impact of aging upon the stress response, we have far less understanding of the transformative period that arises during middle-age. Although it is important to highlight important work done in this area over the past 25 years [19,24,62–64]. Our more recent studies comparing nPM exposure in young (6 month) and middle-age (21 month) male mice found depression of Nrf2 activation and higher levels of its transcriptional repressors, Bach1 and c-Myc in aging [19,24], indicating the deleterious consequences of aging may begin at a much earlier time frame than