Stress Resistance in the Naked Mole-Rat: The Bare Essentials – A Mini-Review

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Naked mole-rat · Longevity · Stress resistance · Nrf2 · p53

Abstract
Background: Studies comparing similar-sized species with disparate longevity may elucidate novel mechanisms that abrogate aging and prolong good health. We focus on the longest living rodent, the naked mole-rat. This mouse-sized mammal lives ~8 times longer than do mice and, despite high levels of oxidative damage evident at a young age, it is not only very resistant to spontaneous neoplasia but also shows minimal decline in age-associated physiological traits. Objectives: We assess the current status of stress resistance and longevity, focusing in particular on the molecular and cellular responses to cytotoxins and other stressors between the short-lived laboratory mouse and the naked mole-rat. Results: Like other experimental animal models of lifespan extension, naked mole-rat fibroblasts are extremely tolerant of a broad spectrum of cytotoxins including heat, heavy metals, DNA-damaging agents and xenobiotics, showing LD50 values between 2- and 20-fold greater than those of fibroblasts of shorter-lived mice. Our new data reveal that naked mole-rat fibroblasts stop proliferating even at low doses of toxin whereas those mouse fibroblasts that survive treatment rapidly re-enter the cell cycle and may proliferate with DNA damage. Naked mole-rat fibroblasts also show significantly higher constitutive levels of both p53 and Nrf2 protein levels and activity, and this increases even further in response to toxins. Conclusion: Enhanced cell signaling via p53 and Nrf2 protects cells against proliferating with damage, augments clearance of damaged proteins and organelles and facilitates the maintenance of both genomic and protein integrity. These pathways collectively regulate a myriad of mechanisms which may contribute to the attenuated aging profile and sustained healthspan of the naked mole-rat. Understanding how these are regulated may be also integral to sustaining positive human healthspan well into old age and may elucidate novel therapeutics for delaying the onset and progression of physiological declines that characterize the aging process.

Introduction

Comparative biology of aging assesses divergent responses in species with disparate longevity and is a useful tool in identifying novel and conserved mechanisms that may contribute to healthspan and longevity, mechanisms that may not be evident using traditional experimentally manipulated animal models. In non-volant, non-aquatic mammals, species longevity is dependent on the size of the organism such that a doubling of species body mass results in a 16% increase in longevity [1]. There are, however, several species that do not conform to this relation-
ship and comparative biology of aging exploits those de-
viant species. While death is not always due to intrinsic
mortality, maximum lifespan potential nevertheless is a
useful indicator of the rate of aging, and species that live
longer than predicted may reveal evolved mechanisms
that delay either the onset or rate of aging. Long-lived spe-
cies are defined as those organisms that live more than
twice as long as predicted by body mass (i.e. have a lon-
gevity quotient $>2$) and are particularly useful in high-
lighting mechanisms that may be involved in retarding
aging processes. Both humans and naked mole-rats (Het-
eroecephalus glaber) are extremely long-lived for their
body size, with longevity quotients ranging between 4
and 10 depending upon the allometric equation used [2].
Although few studies have utilized a comparative ap-
proach in longevity research [3–5], a greater number of
comparative studies are revealing common mechanisms
among long-lived species, which may contribute to sus-
tained good healthspan and long lifespan.

Naked Mole-Rats and Aging Research

The longest-lived rodent known, the naked mole-rat,
has rapidly emerged as a natural model of extreme lon-
gevity based upon its more than 30-year maximum life-
span and prolonged maintenance of positive healthspan
[6]. This $\sim 35$ g rodent lives 8 times longer than similar
sized mice and maintains good health for at least 66% of
its life, which would be equivalent to 80-year-old humans
exhibiting a 30-year-old ‘biological age’ [6].

During their long lifespan, our laboratory has report-
ed that naked mole-rats show a minimal decline in most
aspects of healthspan for at least the first 20 years. They
show unchanged body composition and bone density
with age in addition to minimal changes in physical ac-
tivity and no obvious signs of cognitive decline. Simi-
larly, basal metabolic rate and biochemical activity of
mitochondrial and antioxidant enzyme activity are un-
changed [6].

This species is also able to maintain a healthy repro-
ductive status up until death, with our oldest reproduc-
tive female dying at 27 years of age. Sustained reproduc-
tive capacity and high fertility throughout life provides
further evidence of sustained healthspan as does the
finding that naked mole-rats in captivity show no in-
crease in mortality rate from 2 to 20 years of age. The lack
of an actuarial increase in mortality may reflect the
marked resistance of naked mole-rats to the most com-
mon cause of mortality reported in laboratory rodents,
cancer. In contrast to what has been observed in naked
mole-rats, 70% of one particular laboratory mouse strain
(C57BL/6) mice die of cancer and many more show signs
of lesions and small non-lethal tumors [7]. Further evi-
dence of pronounced cancer resistance was established by
attempts at oncogenic transformation of naked mole-rat
cells. Unlike cells of all other mammals studied to date,
naked mole-rat skin fibroblasts display marked resis-
tance to transformation with Ras$^{G12V}$ and SV40 large T
antigen [8]. Naked mole-rat cells also readily undergo
growth arrest under suboptimal culture conditions
whereas, when maintained optimally, cells grow to high
densities. As such, suboptimal conditions may result in
apparent contact inhibition and this cellular response
may also be integral to their cancer resistance [9]. Both
 genetic and proteomic integrity appear to be maintained
with age, between the ages of 2 and 26 years. Naked mole-
rats show minimal or no differences in a wide array of
biochemical processes and protein homeostasis; this is in
sharp contrast to the age-related differences commonly
seen in laboratory-raised mice [6]. This sustained genom-
ic maintenance may contribute substantially to the ex-
treme longevity of naked mole-rats.

Even at a young age, naked mole-rats have surpris-
ingly high levels of oxidative damage compared to mice.
However, oxidative damage to either lipids or proteins
does not accrue with age in the mole-rats as they do in
aged mice [3]. Clearly, naked mole-rats have mechanisms
in place to protect against stressors and prevent further
oxidative damage accrual and the translation of these
high levels of damage into a decline in normal function
[3].

Naked mole-rats show many common features with
experimental models of long-lived mice (i.e. dwarf and
calorically restricted (CR) mice). Like dwarf mice, naked
mole-rats show decreased body temperature, fasting glu-
cose and metabolic activity as well as enhanced tolerance
of exposure to oxidative stressors compared to similarly
sized wild-type CB6F1 hybrid mice [10, 11]. Multiple
mechanisms have been proposed that may contribute to
the traits evident in models of extended longevity and
many of these may be considered highly adaptive to life
underground.

Naked mole-rats are eusocial mammals, that, like bees
and other social insects, live in colonies of up to 300 ani-
mals, with a strict division of labor culminating in the
presence of a single breeding female who suppresses the
sexual maturity of the subordinates in the colony. Subor-
dinates within the colony maintain the burrow system
and care for young in addition to foraging for food. These
small, pink hystricognath rodents, more closely related to
guinea pigs, porcupines and rock rats than to traditional
laboratory mice and rats, are naturally found in the east-
erern horn of sub-Saharan Africa. Here, they live commun-
ially in an underground maze of tunnels and chambers
up to 8 ft beneath the soil surface [12]. Living underground
protects the mole-rats from extreme changes in weather
and temperature in addition to protection from preda-
tion. However, it poses other challenges associated with
life in a dark dank environment with poor gas and heat
exchange [13]. Not surprisingly, they therefore have low
basal metabolic rates and resting body temperatures [14].

Covered in only sparse, tactile hairs rather than an
insulatory coat of fur, naked mole-rats are thermally la-
bile when housed on their own outside their warm hu-
mid equatorial milieu. Naturally living in large groups
they are also extremely tolerant of hypoxia and hyper-
capnia [13]. Naked mole-rats acquire all their nutrients
and water by feeding off roots, bulbs, corms and tubers
found underground [15]. Often these underground plant
storage components have allelochemicals or secondary
defenses (e.g. cyanogenic glucosides, alkaloids and phe-
nols) to protect themselves against exploitation as food
sources by herbivores. As such, naked mole-rats, like
many other herbivores, may have co-evolved mecha-
nisms to counteract these toxins, in addition to their tol-
erance of hypercapnia and thermodalibity. These are also
integral to their extraordinary longevity.

Multiple mechanisms have been hypothesized for the
large disparity in longevity relative to body size and over-
all health of naked mole-rats. We will evaluate the con-
tribution of cytoprotective mechanisms and stress resis-
tance to aging, healthspan and longevity, of a wide variety
of species with disparate longevity, focusing particularly
on the naked mole-rat.

**Stress Resistance and Aging**

Aging is a multicausal process with conserved charac-
teristics across a variety of species that leads to increasing
susceptibility to both endogenous and external challen-
ges. These include a decline in genomic integrity, an ac-
cumulation of oxidative damage, increases in various pa-
thologies and, most importantly, an increase in mortality
[16]. Perturbation of multiple physiological mechanisms
contributes to the key characteristics that define the aging
profile. These include less efficient DNA repair mecha-
nisms, increased free radical production and oxidative
stress, as well as a decline in proteomic function and in-
tegrity. Oxidative stress is widely regarded as causally
linked to the aging process and is also implicated in many
age-associated diseases such as neurodegeneration, can-
cer and cardiovascular disease, and ultimately, death.

A great deal of published data supports the oxidative
stress theory of aging [17], although recent research fo-
cusing in particular on genetically manipulated mice that
either over- or underexpress key antioxidants has raised
doubts over the importance of reactive oxygen species
and their metabolism as a determinant of longevity [18].
Comparative data across several vertebrate phyla also
yield equivocal support for this theory. Data from birds
and reptiles, as well as long-lived rodent models, show
higher levels of free radical production or oxidative dam-
age accrual, when compared to shorter-lived species. This
does not appear to impact their maximum species life-
span or their healthspan [3].

Species differences in resistance to oxidative and other
endogenous and/or environmental stressors correlate
with maximum species lifespan potential. Enhanced stress
resistance has been shown in skin fibroblasts from
multiple long-lived species after a variety of toxic insults,
including heavy metals, DNA-damaging agents, heat and
dietary interventions [4, 5, 11]. This type of cytoprotec-
tion moves beyond neutralization of oxidative stress but
also encompasses other exogenous chemical stressors
and supports the premise that the degree of endogenous
oxidative stress is not a determinant of species longevity.
Rather, longevity is influenced by how the species re-
ponds to mitigate the impact of that particular stressor.

Considerable evidence has now accumulated showing
that cytoprotection and stress resistance may be a sig-
ificant contributing factor to a positive healthspan and
extended lifespan. Indeed experimental studies of ex-
tended longevity, based upon both genetic manipulations
[19] and dietary restriction [20], together with compara-
tive studies among species with disparate longevity [4, 5,
11] have all revealed that resistance to stressors is a com-
mon trait of longer-lived organisms.

Stress resistance is rapidly emerging as a prominent
predictor of both good healthspan and longevity. Intu-
itively, an increase in stress resistance at a cellular and
organismal level would not only result in augmented re-
sistance to environmental toxins, but also enhanced pro-
tection against inflammation, neurodegeneration and
cancer, diseases that are often associated with aging. In-
creased toxin resistance may also contribute to a more
stable proteome and genome, thereby retarding senes-
cence and aging. Not surprisingly, data published in both
invertebrates and vertebrates show that increased stress

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resistance has a positive relationship with long lifespan [4, 11, 21–24]. The presence of regulated biochemical mediated protection is also evident in that in vitro cytoprotection can be attained by incubating multiple cell types with serum from CR rhesus macaques [25] or with serum from CR humans [20]. In the latter study, cells from mice showed a 25% increase in heat resistance and this was attributed to molecular chaperones present in the serum.

A correlation among in vitro stress resistance and species longevity is also observed in fibroblasts from all phylogenetic groups studied to date, including birds [24], bats [26], domesticated mammals and a diverse array of rodents [4, 5, 11]. Stress resistance is broad-based and not restricted to oxidative stressors including diquat and hydrogen peroxide, but extends to heavy metals and chemotherapeutic agents that directly damage DNA as well. Resistance to stressors in long-lived species has been used as an exemplar of the somatic theory of aging positing that longer-lived species have invested considerable resources in mechanisms to better protect and maintain their somatic tissues than do shorter-lived species [4, 27]. Pro-mislow [28], in re-evaluating published data, observed that the correlation in cellular tolerance of toxins with species longevity was primarily due to body size differences. As many of these studies are performed using fibroblasts in vitro, observed interspecific differences may also be due to divergent optimal culture conditions for the various species. A previous study assessed stress resistance of naked mole-rat cells when both mouse and naked mole-rat fibroblasts were maintained at 33°C, approximating the preferred body temperature of a naked mole-rat, and under serum-starved conditions. This study under suboptimal conditions for the heterogeneous four-way cross mouse cell line revealed that naked mole-rat cells are more resistant to different types of stressors including cadmium, heat, oxidative stress (e.g. diquat), the DNA-damaging agent methyl methanesulfonate (MMS) and starvation [11]. We have extended this study to include assessments under a variety of conditions (e.g. in complete media), including those more favorable for mouse cells and in response to additional toxins, and found that naked mole-rats consistently maintain better protection against a broad array of toxins (table 1), independent of culture conditions.

### Naked Mole-Rats and Stress Resistance

Current data from our laboratory show that the LD$_{50}$ from naked mole-rat fibroblasts is 2- to 20-fold higher than fibroblasts from mice after 2–6-hour treatments with heavy metals and DNA-damaging agents (table 1). Not only are these LD$_{50}$ values higher, but, under conditions considered more optimal for mouse cells than naked mole-rat cells, naked mole-rat fibroblasts still show superior resistance to toxins.

#### Culture Conditions and Toxicity Studies

Toxicity studies have demonstrated that culture conditions, especially prior to toxin treatment, can influence in vitro cellular resistance [29]. Minimum essential medium has been shown to better protect growth-sensitive cell types against oxidative stress [30]. We have specifically conducted our studies in minimum essential medium, which would eliminate potentially deleterious effects resulting from supraphysiologically nutrient-rich media.

We have found that skin fibroblasts of naked mole-rats grow significantly better at 32°C than at 37°C. This is most likely attributed to the low resting body temperature of ~32°C observed in both field-caught and laboratory-maintained animals [14]. We have compared both inter- and intraspecific differences in cell viability in response to heavy metal toxicity at both 32 and 37°C. Increasing the temperature increases the sensitivity to metal toxicity, such that the mouse fibroblast LD$_{50}$ with cadmium declines 2.79-fold when maintained at a 5°C

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**Table 1.** Fibroblasts from the longest-lived rodent, the naked mole-rat, are significantly more resistant to a multitude of stressors in vitro including heavy metals and chemotherapeutic agents, compared to those from the similarly sized but shorter-lived C57Bl/6 mouse.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mouse (LD$_{50}$)</th>
<th>Naked mole-rat (LD$_{50}$)</th>
<th>Fold increase (from mouse to naked mole-rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>12.5</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>Zinc</td>
<td>12.5</td>
<td>30</td>
<td>2.4</td>
</tr>
<tr>
<td>Chromium</td>
<td>5</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>1.25</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Camptothecin</td>
<td>2</td>
<td>30</td>
<td>15</td>
</tr>
</tbody>
</table>

LD$_{50}$ values presented in this table are from fibroblasts maintained in MEM to which 10% fetal bovine serum was added and incubated at 37°C. Cells were exposed to the heavy metal (cadmium, chromium and zinc) for 2 h and to the chemotherapeutic agent (camptothecin, adriamycin) for 6 h. Viability was measured 24 h later by MTT reduction and quantification at 550 nm. Absorbance from untreated controls was set to 1.0; absorbance from treated groups was then calculated as (relative) viability. These data show that NMR cells are more resistant to this diverse set of cytotoxins for the LD$_{50}$ values for naked mole-rat cells range between 2.4- and 16-fold higher than those of mouse cells.
higher temperature and treated in complete medium. In contrast, the naked mole-rat LD50 values are unaffected by the elevation in incubation temperature and, in keeping with their thermal tolerance of a wide variety of ambient conditions, naked mole-rat fibroblasts generally maintain superior resistance to metal toxicity compared to those of mice. Interspecies differences in response to chromium and cadmium exposure (fig. 1) are attenuated when cultivated at the lower temperature. Particularly, mouse fibroblasts show greater resistance to heavy metal toxicity when maintained at the non-physiological, lower ambient temperature compared to mouse fibroblasts cultured at 37°C. Like all biochemical reactions, temperature influences the uptake and metabolic processing of toxins and it is likely that mouse cells, in keeping with their lower metabolic rate induced by the lower temperature, have reduced metal uptake at 32°C and that this contributes to the observed improvement in cell viability. Comparative studies that fail to properly control for optimal cultivation may thus yield spurious findings.

Cultivation of fibroblasts in low serum media prior to metal exposure dramatically reduces the cell viability in a temperature-specific fashion (fig. 1). Serum contains a multitude of proteins important for stimulating growth and attachment of cells and has been shown to modulate the rate of free radical production in porcine lung fibro-
blasts and aortic endothelial cells, affecting their overall maximum population doubling attainable in vitro [31]. Previous reports show that media containing serum provided greater cytoprotection than low serum media against metal toxicity and that serum deprivation is actually considered a source of cellular stress [32]. In only one case, the assessment of chromium toxicity, serum deprivation on mouse fibroblasts had a beneficial effect on survival against metal toxicity (increasing the LD$_{50}$ 3.25-fold, from 5.569 to 18.11 μM; fig. 1). These findings suggest that serum deprivation is not critical for evaluating comparative stress resistance among species.

Overall, our results in low serum media are consistent with those previously published using cadmium [11], in that the naked mole-rat fibroblast LD$_{50}$ is double that observed in mice for cadmium toxicity (fig. 1). Our study reveals that cultivar conditions can greatly influence the LD$_{50}$ toxin concentration. This last point is particularly evident if one compares the resistance to cadmium toxicity of Snell dwarf mice published in two separate studies [19, 33]. Both studies found that fibroblasts isolated from long-lived Snell dwarf mutant mice were about 3-fold greater than wild-type littermates, however, interstudy differences in the recorded LD$_{50}$ values differ by a factor of 5.1. Differences in experimental methodology (i.e. culture conditions) most likely factored into the wide range of values reported and a lack of standardized culture conditions can often hinder interstudy comparisons in a quantitative manner.

Our data reveal that despite the impact of ambient temperature on cell membrane and metabolic properties, as well as the fact that mouse cells were maintained at optimal cultivar conditions whereas naked mole-rat cells at 37°C were not, naked mole-rat cells nevertheless were significantly more resilient to cytotoxic stressors than were mouse cells at 37°C (fig. 1). These findings suggest a broad-based resilience to both heavy metal toxicity and environmental conditions.

Potential Mechanisms of Stress Resistance

Clearly, naked mole-rats must have better cellular protective and/or cell cycle regulation mechanisms than do mice in order to facilitate the observed generalized resistance to stressors. Following treatment with high doses of the heavy metal chromium, the majority of surviving fibroblasts from mice enter senescence. In contrast, fibroblasts from naked mole-rats show considerably less staining with β-galactosidase. If one assumes that this is not due to species differences in responsiveness to β-galactosidase, but rather a true indication of irreversible senescence, this would indicate that fewer naked mole-rat cells irreversibly senesce. This would further support the premise that naked mole-rat cells are more resilient than mouse cells to unfavorable conditions.

We have also evaluated whether or not surviving cells of both species following a cytotoxic insult showed similar proliferative capacity. To assess this we treated the cells with bromodeoxyuridine (BrDU). This synthetic thymidine analog is incorporated into a cell’s DNA during the S-phase of the cell cycle when the cell is dividing and is routinely used as a marker of cell proliferation both in vitro and in vivo [34]. Although mouse cells exhibit extensive early cell death, those that do survive rapidly re-enter the cell cycle and continue to proliferate. This finding suggests that mouse cells are likely replicating before complete repair of damage has occurred (fig. 2). In contrast, even at low concentrations of toxins, at which the majority of naked mole-rat cells survive the insult, the proportion of naked mole-rat cells in S-phase (% BrDU incorporation; fig. 2c) is low. This suggests that those naked mole-rat cells that do survive undergo a prolonged cell cycle arrest in response to genotoxins, and repair damage to their DNA before re-entering the cell cycle, presumably with minimal DNA damage and a reduced chance of mutation induction.

Naked mole-rat fibroblasts exhibit 50-fold higher levels of p53 even under apparently normal growth conditions (fig. 3a). Nevertheless, p53 activity is highly responsive to genotoxic stressors (fig. 3b). For example, when p53 luciferase reporter activities in mouse and naked mole-rat fibroblasts are compared, naked mole-rat cells show a 15-fold increase compared to basal levels whereas only a modest increase (up to 5-fold) was observed in mouse fibroblasts (fig. 3c, d). This rapid increase in p53 may reveal enhanced sensitivity in cell-cycle regulation. Constitutive p53 protein levels are at least 50-fold higher in naked mole-rat fibroblasts compared to those in mice. Thus, even though naked mole-rat cells are capable of long-term proliferation in culture, they are poised to undergo growth arrest via p53 and possibly retinoblastoma protein when they undergo minimal damage. Moreover, we have experimentally shown that if cells in the naked mole-rat fibroblast population do acquire mutations that may be oncogenic, they have yet another protective mechanism; cells enter crisis rather than acquire neoplastic properties (anchorage independence and growth in immunodeficient mice) [8]. We have also found that proteins in tissue homogenates are resistant to unfolding stressors such as heat, urea and iron ascorbate [35], suggesting that enhanced molecular chaperone activity may also be involved
in the observed profile of superior stress resistance in naked mole-rats. Molecular chaperone activity as well as detoxification processes are regulated by the Nrf2-Keap1-signaling cytoprotective pathway [36] and we hypothesize that this pathway is upregulated, largely contributing to the profound stress resistance of the naked mole-rat.

Nrf2 is a transcription factor ubiquitously expressed in all tissues of an organism. Under non-stressed conditions, Nrf2 is bound to Keap1, which with the help of Cullin-3, targets Nrf2 for ubiquitination and subsequent degradation via the proteasome. The Nrf2 half-life under basal (non-stressful) conditions is generally short (~15 min). However, upon a stressful event to the cell, cysteine sensors in Keap1 are modified, altering the conformation of Keap1 and releasing Nrf2. Nrf2 is able to translocate into the nucleus and bind to the antioxidant response element (ARE) to activate the transcription of over 200 cytoprotective genes which include an array of detoxification and
cytoprotective molecules with functions ranging from glutathione and drug metabolism, proteome maintenance and cell cycle regulation. Under stressful conditions, the half-life of Nrf2 is greatly increased (60 min). It is important to note, however, that under basal conditions, some Nrf2 does exist in a free form and is able to constitutively upregulate ARE-activated transcription [37].

Nrf2 has been studied extensively with regard to toxicity [36], cancer [38] and neuroprotection [39] and intuitively should be involved in aging, however research in this area has to a large extent been overlooked. Nrf2 knockout (Nrf2 –/–) mice are more susceptible to induced cancers, neurodegeneration, inflammation, lung and gastrointestinal injury. Fibroblasts and MEFs from Nrf2 –/– mice are also highly susceptible to many different toxins and stressors in vitro [37].

Evidence has been published in an array of animal models that Nrf2 contributes the stress resistance associated with induced models of longevity. Genetic manipulations of Nrf2 homologues in worms (Skn-1) and flies modulate lifespan and contribute to the lifespan-elongating effects of CR [40]. Similarly, multiple mouse models of longevity show an increase in Nrf2 signaling. CR, methionine-restricted and dwarf mice show an increase in Nrf2-dependent molecules, particularly GSH metabolism [41]. GSH-related enzymes involved in synthesis and drug metabolism, in addition to Nrf2, have also been shown to decrease in mice and rats as they age [42]. Interestingly, a mutation that resulted in a cytotoxic effect resulted in upregulation of the Nrf2-signaling pathway and an extension of lifespan in C57BL/6 mice [43]. Although currently there are no lifespan data on Nrf2 –/– mice, one could predict that their lifespan would be shortened compared to wild-type littermates.

Much of the data we find in naked mole-rats is consistent with data regarding Nrf2 in other models of healthy aging and extended longevity. We observe that naked mole-rat fibroblasts have 3-fold higher levels of Nrf2 in non-stressed animals compared to fibroblasts from mice, in addition to similarly elevated levels in Nrf2-regulated enzymes (fig. 4a–d). Nrf2 has been implicated in proteome maintenance, specifically by regulating subunits of the 26S proteasome and thereby facilitating ubiquitinated protein degradation as well as through its regulation of the sequestosome (p62), a protein involved in mediating organelle and protein degradation by autophagy. Both these processes are upregulated in the naked mole-rat and may contribute to highly efficient quality control of the proteome. Not surprisingly, given the high clearance rates of proteins that do not pass quality control, naked mole-rats have low levels of ubiquitinated proteins in their tissue homogenates [35]. The impeccable proteome maintenance that we previously observed thus may be largely due to the increased Nrf2 levels found in the mole-rats.

The superior resistance to a wide array of toxins we observe in fibroblasts isolated from naked mole-rats, in

Fig. 3. Tumor suppressor p53 protein levels, measured by Western blot analyses, are ~50-fold higher in naked mole-rat fibroblasts compared to those from mice (a, b). Naked mole-rat fibroblasts expressing the p53 reporter show enhanced sensitivity to high doses of adriamycin, whereas mouse fibroblasts show a more modest increase in p53 activity 24 h after lower doses of chromium and adriamycin treatment (c, d).
addition to preliminary results we have obtained in vivo, are most likely largely due to elevated Nrf2 signaling as well [44]. These trends are also magnified in vivo, as we have found naked mole-rats to be substantially more resistant to multiple hepatotoxins [unpubl. data], most likely due to their enhanced p53 and increased Nrf2 levels. Decline in vascular health often associated with age appears to be largely attenuated in the naked mole-rat in addition to signs of upregulated defense mechanisms against oxidative stress.

Nrf2 and p53 have also been shown to have a regulatory relationship during cell cycle response to stress [45]. p53 has been shown to inhibit Nrf2 transcription of genes in the ARE after a DNA-damaging event in the cell [46]. The two molecules appear to work together in the prevention of cancer. While the exact mechanism of this interaction is unknown, it is hypothesized that p21 plays a role as well [47]. Particularly interesting to us is that the naked mole-rat, an organism that is highly resistant to spontaneous tumors, has much higher levels of constitutively expressed p53 and Nrf2 compared to the shorter-lived and cancer-prone mouse, thus providing additional, albeit circumstantial evidence that both molecules may act in tandem to regulate the cell cycle, particularly after a toxic insult and thereby protect against cancer.

In summary, cytotoxic stress resistance has begun to emerge as a key indicator of positive healthspan and prolonged longevity. Many of these cytoprotective pathways are highly conserved across many species. In particular, the Nrf2-signaling pathway has now been implicated in longevity and healthspan in evolutionary diverse phyla and may be central to the maintenance of good health well into old age.

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