Research Article

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Cellular stress responses of long-lived and cancer-resistant naked mole-rats [Kansere ve yaşlanmaya karşı üstün direnci bulunan kör farelerin hücresel stres yanıtlarının araştırılması]

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Abstract

Objectives: Some organisms are long-lived and naturally resistant to cancer such as naked mole-rats (NMRs). Studies have shown that these animals can better tolerate stress due to mechanisms, such as upregulation of antioxidant pathways and improved proteostasis. In the present study, we aimed to analyze the tolerance against stress and the cellular mechanisms related to the stress response in NMR cells comparative to mouse cells.

Materials and methods: NMR and mouse fibroblasts were exposed to cellular stresses including H_2O_2 and/or $NaNO_3$ and the viability of the cells were analyzed. In addition messanger RNA (mRNA) expression of antioxidant transcription factor Nuclear factor erythroid-derived 2-like 2 (Nrf2) and its target gene NAD(P)H quinone dehydrogenase 1 (NQO1) were determined by qRT-PCR and comprehensive analysis of stress-related gene expression was performed by RNA-Sequencing in fibroblasts and induced pluripotent stem cells (iPSC).

Results: Surprisingly, NMR fibroblasts were found to be more sensitive than mouse cells to H_2O_2 and $NaNO_3$. Furthermore, it was shown that fibroblasts and iPSCs mainly aren't identical in the expression pattern of cellular defense signaling and several factors are mainly downregulated in NMR iPSCs.

Conclusions: Collectively, the data gained from the present study help to improve the understanding of evolved mechanisms that contribute to stress resistance, aging and cancer.

Keywords: aging; cellular signaling; iPSC; naked mole-rat; nitrosative stress; oxidative stress; stress resistance.

ÖZ

Amaç: Çıplak kör fareler (ÇKF) sıra dışı uzunluktaki ömür süreleri ve kansere karşı dirençli olmaları ile dikkat çekmektedirler. Yapılan çalışmalar, bu hayvanların antioksidan yolaklar ve proteostasis gibi mekanizmalarla strese karşı üstün dirençleri olabileceğini göstermektedir. Bu sebeple çalışmanın amacı, çıplak kör farelerin strese karşı dirençlerinin ve stres yanıtında rol oynayan temel mekanizmalarının laboratuvar farelerine karşı kıyaslanarak araştırılmasıdır.

Gereç ve Yöntem: ÇFK ve fare fibroblast hücreleri, H_2O_2 ve/veya $NaNO_3$ uygulamaları ile hücresel strese maruz bırakıldı ve hücrelerin canlılığı analiz edildi. Buna ek olarak, Nrf2 ve hedef geni olan NQO1 mRNA ekspresyonları qRT-PCR tekniği ile belirlendi. Stres ile ilişkilendirilen temel mekanizmaların daha kapsamlı gen ekspresyonu analizinin yapılması için fibroblast ve indüklenmiş kök hücreleri (iPSC) RNA-sekanslama tekniği ile analiz edildi.

Bulgular: Elde edilen bulgular ilginç bir şekilde ÇKF fibroblast hücrelerinin farelere göre H_2O_2 ve $NaNO_3$ stresine karşı daha hassas olduğunu gösterdi. Bunun yanında, hücresel savunma mekanizmaları açısından fibroblast ve iPSC hücrelerindeki ekspresyonların paralel olmadığı ve bir çok faktörün ÇFK İPSC'lerinde azaldığı gözlendi.

Sonuç: Elde edilen bu sonuçlar, yaşlanma ve kansere karşı geliştirilen stres direncinin aydınlatılmasına katkı sağlamaktadır.

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Anahtar Kelimeler: Yaşlanma; çıplak kör fare; stres direnci; iPSC; nitrosatif stres; oksidatif stres; hücre sinyal yolakları.

Introduction

Life expectancy has increased highly in the last half a century and people now spend more of their lives prone to age-related diseases. Aging is a process accompanied by a progressive loss of physiological integrity, the increased risk for diabetes, cardiovascular and neurodegenerative diseases, cancer and other chronic diseases, and most importantly, increased mortality [1]. However, the molecular mechanisms of aging are not still well-defined.

Some organisms are long-lived and naturally resistant to age-related diseases. Of these, naked mole-rats (NMR, *Heterocephalus glaber*), the longest-lived rodent known, have more than 30-year maximum lifespan and prolonged maintenance of positive healthspan [2]. This rodent lives eight times longer than the similar sized house mice (*Mus musculus*) and spends most of its life as healthy as 30-year-old 'biological aged' human [3]. Moreover, NMRs are also shown to be highly resistant to both spontaneous and experimentally induced cancer [4]. Previously the unique genome of NMR was sequenced and demonstrated molecular adaptations related to their superior cancer resistance [5]. Other biomedical features of NMRs include their resistance to hypoxia conditions. A very recent study revealed that these animals were able to survive in anoxic conditions for 18 min without any significant injury [6]. As a result, NMR has been accepted as a natural model of aging and cancer, therefore, is a useful model in medical studies [3].

Oxidative and other stresses are widely linked to the aging process and are also implicated in age-related diseases. Previous studies have demonstrated that cytoprotection and stress resistance significantly contribute to a positive healthspan and extended lifespan [7]. Comparative studies among species have revealed that resistance to stressors is a common feature of longer-lived organisms including NMRs [8, 9]. NMR has also been a very interesting organism to study the effect of stress since it has been shown to have slowed metabolic and respiratory rates. More importantly, their protein structure and function were not affected by oxidative stress during aging [10].

Among antioxidant signaling pathways, Nuclear factor erythroid-derived 2-like 2 (Nrf2), a key antioxidant transcription factor, has been proposed as one of the promising mechanism to explain the stress-resistance of NMRs [8, 9]. Activation of Nrf2 protein increases the transcription of antioxidant response-element genes, proteasome subunits, heat shock proteins (Hsp), all of which protect the cells against damage caused by oxidative stress [11, 12]. Nrf2 has been studied extensively in diseases such as cancer [13], atherosclerosis [14] and neuroprotection [15] and intuitively should be involved in longevity.

The aim of the present study is to investigate the mechanisms that are implicated in contributing to stress resistance and longevity in NMR cells. In this regard, a particular opportunity is provided by induced pluripotent stem cells (iPSCs) since they have limitless proliferation capacity and a potential to differentiate into embryonic lineages [16]. iPSC model is rapidly being used as a major tool to understand and cure age-related diseases, therefore, analysis of NMR iPSCs could bring a much-needed resource to characterize relevant phenotypes [2]. Therefore, in the present study, we analyzed Nrf2 target genes in NMR fibroblast and iPSCs in comparison to mouse cells to find out the possible new roles Nrf2 signaling.

Materials and methods

Isolation and cell culture of embryonic fibroblasts

C57BL/6 mouse and NMR cells were isolated as previously described [2, 17] and ready to use for the present study. Briefly, the mouse embryos were harvested from mouse fetus on day E13 and were dissected, excluding the internal organs and brain. Then, the fetus was washed in phosphate saline buffer (PBS), cut into the small pieces and digested in 0.25% trypsin-EDTA solution. For the inactivation of trypsin, Dulbecco's Modified Eagle's medium (DMEM) (Thermo Fisher Scientific, Germany) with 20% fetal bovine serum (FBS) was added and tissues were pipetted up and down to generate the cell suspension. Mouse embryonic fibroblasts (MEF) were then cultured in DMEM (DMEM high glucose + GlutaMAX) (Thermo Fisher Scientific) with 10% FBS, penicillin/streptomycin (10,000 U/mL) (Thermo Fisher Scientific), 1× non-essential amino acids (Thermo Fisher Scientific), 0.1 mM β-mercaptoethanol were cultured at 37 °C and 5% CO_2.

NMR embryonic cells were isolated as MEFs and the difference is explained as follows; NMR embryos were taken at the early stage of fetogenesis, ~45 days postcoitum. NMR fibroblasts were incubated in DMEM (DMEM high glucose + GlutaMAX) with 15% FBS, penicillin/streptomycin (10,000 U/mL), 1× non-essential amino acids, 0.1 mM β-mercaptoethanol. Isolated embryonic NMR fibroblasts were cultured under hypoxic and hypothermic conditions; at 32 °C, 3% O_2, and 5% CO_2.

H_2O_2 and $NaNO_3$ treatment and viability assay

NMR and mouse embryonic fibroblasts were plated in 96-well plates to induce stress conditions by H_2O_2 or/and $NaNO_3$. 1×10^4 cells/well were plated and incubated for 48 h. Fresh 1–10–100–500 μM H_2O_2 solutions were prepared from 100 mM stock solution. Fresh 10–25–50–75 mM $NaNO_3$ solutions were diluted from 1 M stock $NaNO_3$ solution. Cells were treated with H_2O_2 or/and $NaNO_3$ in 100 μL of culture medium and incubated for 24 h. After 24 h, 20 μL MTS solution (Promega, USA) was added to the wells and incubated for 2 h. Viability was measured at 490 nm

using a 96-well plate reader. For the calculation of viability, blank was extracted from the raw data and normalized against control wells.

Mouse and NMR fibroblast reprogramming to iPSC

NMR and mouse fibroblasts were reprogrammed as previously described [2, 17]. Briefly, fibroblast cells were transduced with lentivirus that contained polycistronic mouse OSKM and FUW-M2rtTA vectors [18]. Viral supernatants added to the cells for two times every 24 h. After three days' incubation, infected cells were seeded on mytomycin C (MilliporeSigma, Germany) inactivated MEFs. N2B27 + 2i reprogramming medium containing 2 μg/mL doxycycline (MilliporeSigma) was used. Culture media was changed with a 24 h interval. When the embriyonic stem (ES)-like colonies were generated, they are maintained on feeder in N2B27 + 3i medium. Cells were cultured for six days and doxycycline was removed after passage 5 [2].

RNA isolation for quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA from fibroblasts and iPSCs were isolated by RNA Mini Kit (Qiagen, Germany) and the amount and purity of RNA extracts were measured via Qubit Fluorometer (Thermo Fisher Scientific). Complementary DNA (cDNA) was synthesized with using 100 ng total RNA and iScript cDNA synthesis kit (Bio-Rad, USA) and qRT-PCR was performed QuantiTect PCR Sybr Green kit (Bio-Rad). The samples and the standard curve were generated simultaneously in one run, and the results normalized to 40S ribosomal protein S18 (Rps18) messanger RNA (mRNA) expression. Primers were designed for NMR (*H. glaber*) Fasta format of Nrf2, NAD(P)H kinon dehidrogenaz 1 (NQO1) and Rps18 using Primer3 Output software. Primer sequences are shown in Table.

Data analysis of RNA-sequencing

Mouse and NMR fibroblast lines and their respective iPSCs were used for the RNA-Sequencing analysis in the previous study [2] and transcriptomics data re-analyzed for the stress related pathways for the present study. Data analysis is as follows: *M. musculus* (GRCm38) and *H. glaber* (hetGla2) reference genomes and annotation information were extracted from The National Center for Biotechnology Information (NCBI) database. For the differential gene expression analysis, the data was firstly analyzed with FastQC for quality check. Then, raw data were cleaned with Trim_Galore (version 0.4.0) and aligned to reference genomes using TopHat2 and Bowtie softwares. After the alignment, assemble of transcripts were done by using Cufflinks, and feature counts are determined by Python/HTseq. The analysis for differential expression of genes (DEG) was performed by EdgeR and DESeq [2]. The transcriptome data gained from the analysis was used to reanalyze Nrf2 signaling.

Statistical analysis

Data denote measurements were expressed as group means ± SD which were obtained from 3 to 6 different samples. Statistical significance and p-values were determined by one-way analysis of variance (ANOVA) and Tukey's or Newman–Keuls multiple comparisons test using GraphPad Prism 7.0 Software. $p<0.05$ was considered as significant data.

Results

NMR fibroblasts are more sensitive to the stress generated by H_2O_2 and $NaNO_3$

To test the effects of stress conditions, NMR and mouse cells were treated either with H_2O_2 or/and $NaNO_3$ at various concentrations to induce oxidative stress and nitrosative stress, respectively. Interestingly, our results revealed that NMR fibroblasts were more sensitive to both oxidative stress and nitrosative stress (Figure 1). Even low concentrations of H_2O_2 (1 μM) was sufficient to affect the viability of NMR cells. The same pattern was followed by nitrosative stress treatment and 25 μM $NaNO_3$ was found to have dramatic effects on the viability of NMR cells. Previous studies are also consistent with our data and showed that NMR fibroblasts were almost four times more sensitive to several stresses such as H_2O_2, endoplasmic reticulum (ER) stress, UV light and rotenone when compared to mouse fibroblast [19]. However, the effect of nitrosative stress has been shown for the first time in the present study.

Nrf2 and NQO1 mRNA expression in fibroblasts and iPSCs

Our findings on the stress response of NMR cells triggered us to study signaling of stress pathways in detail. Nrf2 is a

Table 1: The sequences of primers designed for Nrf2, NQO1 and Rps18 in mouse and NMR.

Gene name	Forward (F) or Reverse (R)	Mouse	NMR
Nrf2	F	AGTCCCAGCATAGCATCACC	CTCGCTGGAAAAAGAAGTGG
	R	ACAGAGGTTGCACGGTATCC	CCGTCCAGGAGTTCAGAGAG
NQO1	F	CCGTAGAGGTGCTGAAGAGG	TTCTCTGGCCGATTCAGAGT
	R	TGCAGCTTCCACCTTCTTTT	GGCTGCTTGGAGCAAAATAG
Rps18	F	ATCCCTGAGAAGTTCCAGCA	TGTGGTGTTGAGGAAAGCAG
	R	CCTCTTGGTGAGGTCGATGT	TCCCATCCTTCACATCCTTC

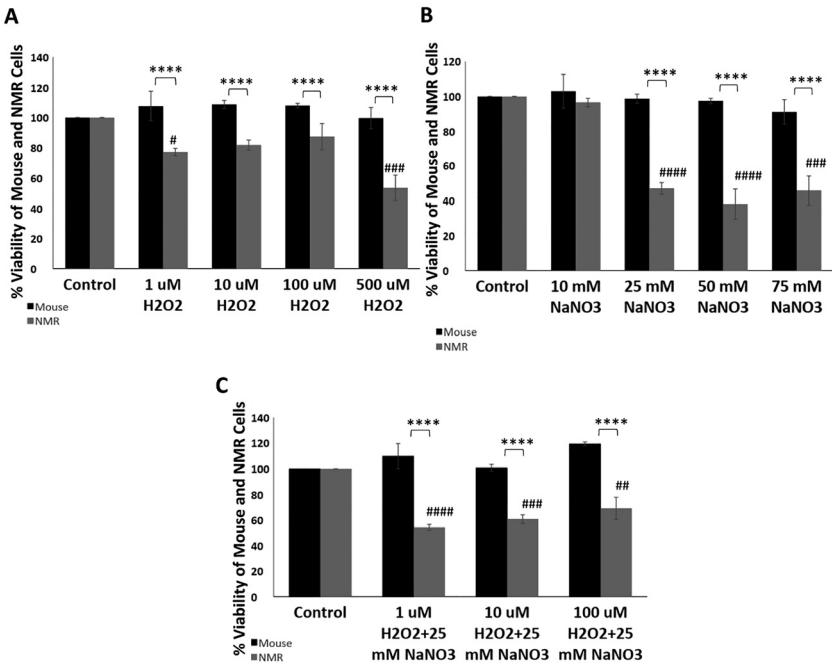

Figure 1: Viability percentage for mouse and NMR cells following H_2O_2 and $NaNO_3$ exposure.
Cells were treated with H_2O_2 and/or $NaNO_3$ for 24 h and cell viability was analyzed by MTS assay. (A) Mouse and NMR fibroblasts are treated with 1–10–100–500 μM H_2O_2, ****p<0.0001, #p=0.0366 and ###p=0.0003 vs. NMR control (B) Mouse and NMR fibroblasts are treated with 10–25–50–75 mM $NaNO_3$, ****p<0.0001, ####p<0.0001 vs. NMR control (C) Mouse and NMR fibroblasts are treated with 1–10–100 μM H_2O_2 and 25 mM $NaNO_3$, ****p<0.0001 and ####p<0.0001 vs. NMR control, ###p=0.0005 vs. NMR control, ##p=0.0054 vs. NMR control. Data are expressed as mean ± S.E. (n=3).

transcription factor that regulates the expression of many cellular defense players including antioxidants, heat shock proteins and proteasome subunits [9]. Previous studies have hypothesized Nrf2 signaling as the responsible pathway for the extraordinary lifespan of the NMR [8, 9]. Firstly, we tested Nrf2 and its target gene, NQO1 mRNA levels in both fibroblasts and in iPSCs to compare mouse and NMR expressions (Figure 2). We found that both Nrf2 and NQO1 mRNA expressions were elevated in NMR fibroblasts when compared to mouse fibroblasts. On the other hand, the expressions of NQO1 were reduced both in NMR and mouse iPSCs in comparison to their respective fibroblasts indicated a common cell-type specific expression of NQO1.

Comprehensive gene expression analyses of Nrf2 pathway in mouse and NMR cells

To gain insights into gene expressions related to stress response in detail, we analyzed RNA-Seq data of fibroblasts and their respective iPSC lines. To clarify the role of Nrf2-signaling, DEG analysis of RNA sequencing focused on genes that are the targets of Nrf2 signaling (Figures 3–5). Our data demonstrated that among Nrf2 target genes; glutamate—cysteine ligase catalytic subunit (GCLC), heme oxygenase-1 (HO-1) and Taldo1 RNA expressions were significantly elevated in NMR fibroblasts when compared to mouse fibroblasts. In addition, GCLC expression was reduced in NMR iPSC when compared to mouse iPSC. HO-1

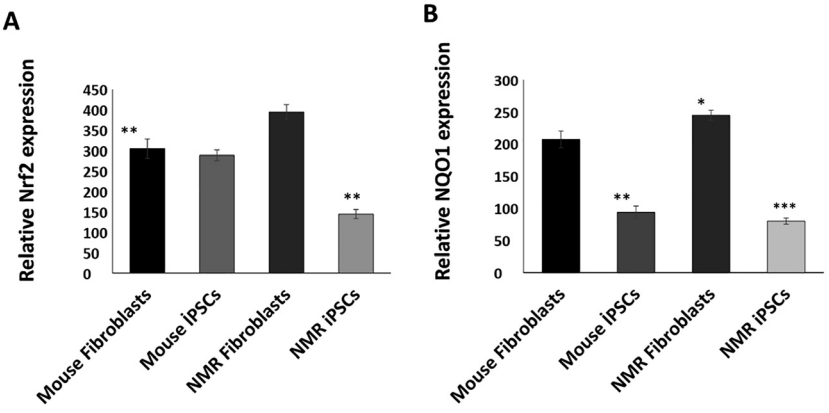

Figure 2: mRNA expressions of Nrf2 and NQO1 in mouse and NMR cells.
Nrf2 and NQO1 mRNA expressions are measured by qRT-PCR and normalized against Rps18. (A) Nrf2 mRNA expression, **p<0.005 vs. NMR fibroblasts. (B) NQO1 mRNA expression, **p<0.005 and *p<0.05 vs. mouse fibroblasts, ***p<0.001 vs. NMR fibroblasts. Data are expressed as mean ± S.D. (n=3).

expressions were found be reduced in iPSC of mouse and NMR when compared to their respective fibroblasts (Figure 3). On the other hand, transketolase (TKT) expression was increased in NMR iPSCs when compared to NMR fibroblasts and mouse iPSCs. However, sulfiredoxin1 (SRXN1) expressions were found be lower in both mouse and NMR iPSCs when compared to their respective fibroblasts. No significant change was shown in glutathione-disulfide reductase (GSR) levels (Figure 4). The genes related to proteostasis were also investigated and it was demonstrated that Hspa4 expression was decreased in NMR iPSCs when compared to mouse iPSCs. Hsp90 expression was elevated in mouse iPSCs when compared to mouse fibroblasts. Finally, Psmc2, a proteasome subunit, expression was reduced in NMR iPSCs when compared to NMR fibroblasts. Our results indicated variance between target genes of Nrf2 in NMR cells suggesting each of the Nrf2 signaling player could play different roles. In addition, our RNA-Seq results were confirmed our qRT-PCR results and we showed that Nrf2 and NQO1 expressions showed the same pattern in both qRT-PCR and RNA-Seq analysis (Supplementary Figure).

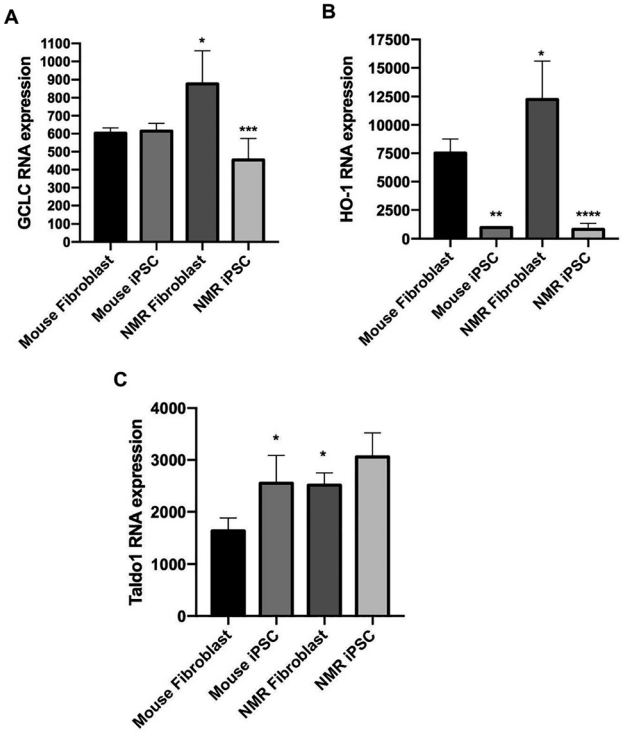

Figure 3: RNA expressions of Nrf2 target genes GCLC, HO-1 and Taldo1 in NMR and mouse cells.
RNA expressions are determined by RNA-Sequencing. (A) GCLC, *p=0.0209 vs. mouse fibroblasts and ***p=0.0009 vs. mouse iPSC. (B) HO-1, **p=0.0013 and *p=0.014 vs. mouse fibroblasts, ****p<0.0001 vs. NMR fibroblasts. (C) Taldo1, *p=0.0291 mouse fibroblast vs. mouse iPSC, *p=0.0369 mouse fibroblast vs. NMR fibroblast. Data are expressed as mean ± S.E. (n=4).

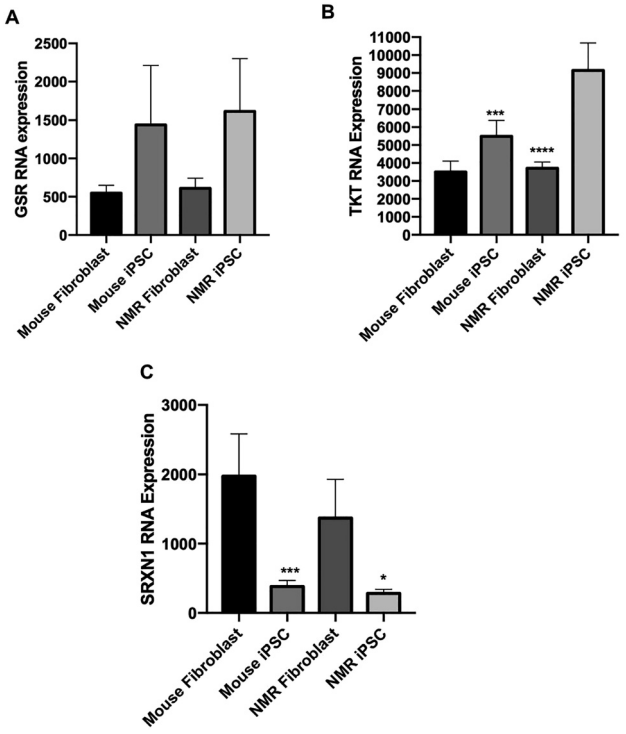

Figure 4: RNA expressions of Nrf2 target genes GSR, TKT and SRXN1 in NMR and mouse cells.
RNA expressions are determined by RNA-Sequencing. (A) GSR, statistically no significant change was found. (B) TKT, ***p=0.0008 and ****p<0.0001 vs. NMR iPSC. (C) SRXN1, ***p=0.0008 vs. mouse fibroblast, *p=0.0141 vs. NMR fibroblast. Data are expressed as mean ± S.E.

Discussion

It has long been suggested that the mechanisms that controls the aging process could be in parallel to the mechanisms related to stress resistance [20, 21]. A supportive study for this hypothesis showed that single gene mutations that results in the extension of *Caenorhabditis elegans* lifespan, also enhances the stress resistance against various stresses such as oxidative stress, UV light, heavy metals and heat [22–25]. Research in mammals also demonstrated similar results such as insulin-like growth factor 1 (IGF-1) mutation which both increases the longevity in mice increases the stress resistance against paraquat, an agent that induces oxidative stress [26–28]. We, therefore, in the present study, aimed to test the stress response and related signaling pathways in the longest-lived rodent, the NMR in comparison to short-lived mice.

NMR and mouse cells treated either with H_2O_2 for oxidative stress or/and $NaNO_3$ for nitrosative stress. Our results revealed that NMR fibroblasts were more sensitive

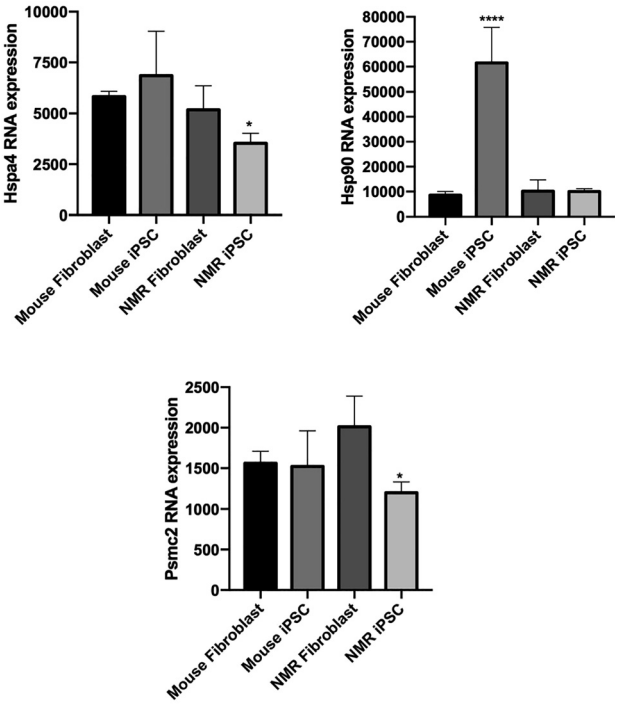

Figure 5: RNA expressions of Nrf2 target genes related to proteostasis.
RNA expressions are determined by RNA-Sequencing. (A) Hspa4 RNA expression, *p=0.0135 vs. mouse iPSC. (B) Hsp90 RNA expression, ****p<0.0001 vs. mouse fibroblasts and NMR iPSC. (C) Psmc2 RNA expression, *p=0.0128 vs. NMR fibroblast. Data are expressed as mean ± S.E.

to both of stress conditions and to our knowledge, this is the first study showing the effect of $NaNO_3$ in NMR cells. It has been previously shown that NMR is more sensitive to some forms of stresses [19]. When NMR cells were compared to mouse cells, NMR cells were shown to be resistant to methyl methanesulfonate, heat, paraquat, cadmium and low-glucose medium, correlated with the idea that cellular resistance to stress may contribute to longevity. However, NMR cells were shown to be more sensitive to several stress types such as ultraviolet (UV) light, H_2O_2, and ER stress [19]. Our results are consistent with the previous data and we also found that NMR fibroblasts were more sensitive both H_2O_2 and as well as, $NaNO_3$, than the mouse fibroblasts. H_2O_2 results in oxidative stress through the production of hydroxyl radicals that damages the membranes, lipids and nucleic acids [29]. Similarly, $NaNO_3$ causes nitrosative stress through the production of nitric oxide that exerts its toxic effects by reacting with many cellular components, including lipids, DNA, metals and thiols which leads to the damage in the cell [30]. One explanation for the loss of cellular resistance in NMR cells to H_2O_2 and $NaNO_3$ could be the evolutionary adaptations to the natural environment of NMRs where the gaseous atmosphere within the burrows is hypoxic and hypercapnic [19].

Nrf2 is a transcription factor that is expressed in all tissues and is active under both homeostatic and stressful conditions [31]. This factor induces the transcription of many molecules including antioxidants, molecular chaperones and proteasome subunits [9]. In turn, it leads to multidirectional protection in toxin eradication and removal of oxidative stress. It also contributes to proteostasis by both HSPs and proteasomal degradation or autophagy for the removal of damaged proteins [11]. Nrf2 also protects against cancer, inflammation and neurodegeneration, and it positively influences both the healthspan and lifespan of an organism [11]. A recent study compared Nrf2 and target gene expressions in NMR and mouse liver and found the significant increase in NMR liver [13]. The same study also compared NMR with nine other rodent species revealing that Nrf2 signaling positively correlates with maximum lifespan. In the present study, mRNA levels of Nrf2 and its target NQO1 were measured in fibroblasts of NMR in comparison to mouse. Our results are consistent with the previous studies and Nrf2 and NQO1 expressions were found to be higher in NMR fibroblasts when compared to mouse fibroblasts. These results indicate that Nrf2 signaling could contribute to the extraordinary health properties of NMRs.

Several studies have been used iPSCs as a substitute for embryonic stem cells (ESCs) and a source of somatic cells. iPSCs generation from NMR is an essential process to target biomedical research and provide cell culture systems for aging and cancer-related research. Therefore, our group generated iPSCs cells from NMRs and observed that these cells had propensity for a tetraploid karyotype and interestingly demonstrated that NMR iPSCs were resistant to forming teratomas [2]. In the present study, mRNA levels of Nrf2 and its target NQO1 were also measured in iPSCs of NMR and mouse in addition to fibroblasts. Surprisingly, it was shown that Nrf2 expression was decreased in NMR iPSCs when compared to NMR fibroblasts and to mouse iPSCs. NQO1 expression was also shown to have very low levels in NMR iPSCs compared to NMR fibroblasts, however this effect was not unique to NMR and the same pattern was also observed in mouse cells. Recent studies have demonstrated the mechanism of Nrf2 signaling and its new functions, apart from its well-known role in the antioxidant response. Previously, Nrf2 was shown to regulate the maintenance of mesenchymal stem cell (MSC) self-renewal and differentiation potential [32]. It was also shown that Nrf2 activation played a role in cell proliferation and the colony forming ability of early passage MSCs [32]. Recently, Nrf2 was demonstrated to regulate self-renewal

and quiescence in hematopoietic stem cells [33]. Therefore, our results suggest that downregulation of Nrf2 and its down-targets could function for the differentiation process of NMR stem cells. On the other hand, resistance of NMR iPSC to form teratoma is clearly distinctive property of NMR iPSCs cells from mouse iPSCs, therefore, decreased activation in Nrf2 signaling in NMR iPSCs could also play a role in this interesting feature of NMR.

Although many genes are regulated in a Nrf2-dependent manner, identification of specific target genes have not been distinguished in NMR cells. We, therefore, reanalyzed NMR RNA-Seq data in order to clarify the target genes of Nrf2 signaling in fibroblasts and iPSCs. Our results showed that, among Nrf2 target genes GCLC, HO-1 and Taldo1 expressions were found to be increased in NMR fibroblasts when compared to mouse fibroblasts. These results may reflect the fact that Nrf2 and these down-targets could be responsible for the superior stress resistance of NMRs, however, may not play a role against the stress induced by H_2O_2 and $NaNO_3$.

On the other hand, SRXN1 expression was found be decreased in both mouse and NMR iPSCs when compared to their respective fibroblasts. Other Nrf2 targets genes including the proteostasis players acted differently in NMR cells. Therefore, our results indicated variance between Nrf2 and related gene expressions between mouse and NMR cells as well as between fibroblasts and their respective iPSCs. These results suggest various Nrf2 signaling players could play separate roles in the stress resistance.

Conclusion

In the present study, it is demonstrated that although NMRs that are known with their stress resistance, are not more resistant to all kind of stresses. Our findings are consistent with the previous studies and their lower resistance seems to be reasoning from their evolutionary adaptations. Moreover, the main stress mechanism and its down-targets were investigated in NMR cells. The elevated expressions of Nrf2 and target genes including NQO1, GCLC, HO-1 and Taldo1 in NMR fibroblast cells suggest that these genes could play a role in the resistance of NMRs to other stressors. In addition, decreased expressions of several Nrf2 down-target genes in NMR iPSCs may indicate a possible role of Nrf2 signaling apart from its antioxidant role in iPSCs. However, further studies are required to confirm our hypothesis, such as the transcriptomics analysis of stressed cells against control cells would better explain the causal mechanisms responsible for stress resistance of NMR.

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Competing interests: There is no conflict of interest to disclose.

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