Preincubation with the Proteasome Inhibitor MG-132 Enhances Proteasome Activity via the Nrf2 Transcription Factor in Aging Human Skin Fibroblasts

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ABSTRACT: Strategies that lead to the upregulation of the proteasome are known to elicit beneficial consequences to the organism by countering oxidative stress–associated disorders, such as protein conformational diseases, cancer, and aging. Mild treatment with proteasome inhibitors has been previously demonstrated to stimulate proteasome activity and cellular resistance against oxidative injury. However, the mechanism for this action has not been clearly defined. We examined the role of the nuclear factor-E2-related factor 2 (Nrf2) in fibroblasts, a key transactivator of the antioxidant response pathway, in the regulation of the proteasome by its inhibitor MG-132. Here, we demonstrate that the stimulation of the proteasome by low levels of MG-132 can be abrogated by small interfering RNAs (siRNAs) targeted against Nrf2. Consistently, cells that constitutively express Nrf2 exhibit elevated levels of proteasome activities. We further investigate how its beneficial effects, that is, proteasome stimulation, are manifested in young and replicative-senescent cells. Our data underscore that manipulation of Nrf2 by the administration of pharmacologically low levels of proteasome inhibitors may prove to be an alternatively potent strategy for inducing long-term protective effects against oxidative stress.

KEYWORDS: Nrf2; proteasome; MG-132; fibroblast; hormesis; aging

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INTRODUCTION

Long-term exposure to proteasome inhibitors leads to severe oxidative stress, cell cycle arrest, downregulation of the proteasome, accumulation of protein aggregates, and appearance of senescence-like phenotypes.[1–5] It is widely accepted that the induction of cell cycle arrest is mainly attributed to the inhibition of the ubiquitin 26S proteasome pathway, which controls cell cycle regulators.[5,8] Analysis of gene expression in neural cells subjected to 12-week proteasome inhibition showed a wide range of cellular changes, such as reduced proteasome expression, and a weakened oxidative defense mechanism accompanied by the downregulation of glutathione S-transferase among others.[6] Such reduction in oxidative defense in response to proteasome inhibition is also evident from the increased levels of DNA and RNA oxidation.[7]

Short-term proteasome inhibition, on the other hand, results in an opposing effect that leads to improved cellular fitness accompanied by the induction of glutathione S-transferase (GST), heat-shock proteins (HSPs), and several proteasome genes.[9] The activation of oxidative-defense genes is known to occur through transcription factors like heat-shock factor 1 (HSF1), activator protein 1 (AP-1), and Nrf2.[9,10] Recently, it has been found that the proteasome is also transcriptionally regulated by Nrf2, suggesting a role for a proteasomal degradation pathway in the cellular adaptive response to oxidative stress.[11] In fact, the promoter regions of 20S and 19S, but not the immunoproteasome, harbor two antioxidant response elements (AREs).[12]

The Nrf2 is a cap-n-collar family of transcription factors that regulates cellular defenses against ROS. Under normal physiologic conditions, it exists in its inactive form because of sequestration by the cytoplasmic nuclear translocation-inhibitor KEAP1, which delivers it to proteasomal degradation.[13–15] Upon oxidative stress, Nrf2 is released from KEAP1 and translocates to the nucleus and initiates transcription of some 200 genes with the *cis*-acting antioxidant response element (ARE). Most of these genes have known roles in protecting the cell against oxidative and electrophilic stressors.[11] As activity of the proteasome is shown to be stimulated by short-term or low-dose exposure to proteasome inhibitors, we wanted to test whether Nrf2 is the transcription factor that controls this negative feedback loop of proteasome activity and gene expression. Furthermore, we tested whether the response to proteasome inhibition differs between young and replicative-senescent human cells.

RESULTS AND DISCUSSION

Normal adult human fibroblasts were co-transfected with the pNQR-ARE, a firefly luciferase reporter construct controlled by an ARE in the promoter region, and pRL-RSV, a constitutive *Renilla* luciferase expression plasmid. After incubation for 12 h with increasing amounts of MG-132 (2.5–30 nM),

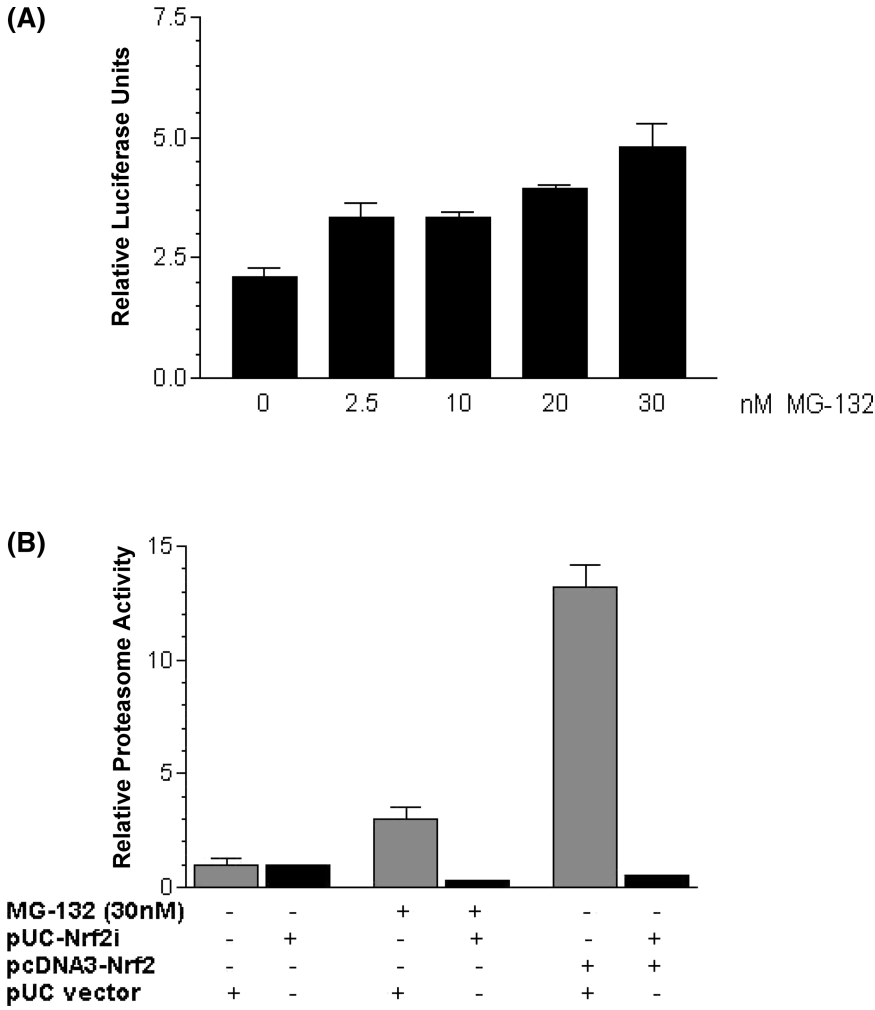

FIGURE 1. Pretreatment with the proteasome-inhibitor MG-132 leads to an increased Nrf2, an upregulated proteasome activity in young cells, which was abolished by Nrf2 siRNA. (**A**) Dual-luciferase reporter (pNQR-ARE, pRL-RSV) on TIG-1 cells treated with different concentrations of the MG-132 for 12–16 h resulted in the upregulation of the Nrf2. (**B**) Assay for the chymotrypsin-like proteasome activity of young cells after treating with 30 nM MG-132 for 16 h and Nrf2 shRNA.

a dual-luciferase assay was performed using a commercial kit from Promega (Madison, WI). The increasing levels of MG-132 resulted in a dose-dependent elevation of Nrf2 activity with the highest induction at 30 nM (FIG. 1A). After 12 h of incubation with 30 nM MG-132, proteasome activity was increased but not to the extent of the cells transfected with the pcDNA3-Nrf2, a

CMV-driven Nrf2 expression cassette (FIG. 1B). The increase in proteasome activity in Nrf2-overexpressing transfectants and in cells treated with 30 nM MG-132 was abolished after (co-)transfection with pUC-Nrf2i, a U6 promoter–driven short hairpin RNA against Nrf2 (FIG. 1B). These results demonstrated that proteasome activity could be stimulated by MG-132 and this proteasome-stimulatory effect may be controlled by the Nrf2 transcription factor.

Finally, we tested the reactivity of young and replicative-senescent cells to mild and severe treatment with MG-132. Proteasome activity was induced by 30 nM (mild) MG-132 treatment both in replicative-senescent (old) and young cells. However, in the old cells induction was only about one-third of the increase in proteasome activity exhibited by young cells. Treatment with MG-132 (30 μM for 1 h) induced severe cell death in the rapidly growing young cells (∼84% death), whereas the replicative-senescent cells were less affected (∼33% death) (data not shown). These data suggest that MG-132-induced protective effects against oxidative stressors via Nrf2 activation may be differentially regulated in young and senescent cells.[16–19]

In summary, our data underscore that the manipulation of the Nrf2 pathway by pharmacologically low levels of proteasome inhibitors could prove to be an alternative potent strategy for inducing long-term protective effects against oxidative stress and aging. We are further investigating how such beneficial effects, that is, proteasome stimulation and increased fitness, are manifested during replicative senescence.

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