MAP Kinases and Heat Shock–Induced Hormesis in Human Fibroblasts during Serial Passaging *in Vitro*

ELISE R. NIELSEN, YVONNE E.G. ESKILDSEN-HELMOND, AND SURESH I.S. RATTAN

Laboratory of Cellular Ageing, Danish Centre for Molecular Gerontology, Department of Molecular Biology, University of Aarhus, Aarhus, Denmark

ABSTRACT: Adult human skin fibroblasts were exposed repeatedly to 41°C or 42°C heat shock (HS) for 1 h twice a week during serial passaging throughout their replicative life span. On the basis of longevity curves, cell size, and morphology, we observed that repeated mild heat shock (RMHS) at 41,°C had strong anti-aging hormetic effects, including 20% extension of cellular longevity. The basal levels of the MAP kinases JNK1, JNK2, and p38 increased during serial passaging, while that of ERK2 decreased. RMHS further exaggerated these effects, which suggests that age-related changes in MAP kinases may be an adaptive response for better cell survival.

KEYWORDS: stress; hormesis; aging; anti-aging; MAP kinases

INTRODUCTION

Anti-aging hormetic effects of repeated mild heat shock (RMHS) on normal human fibroblasts have been reported earlier from our laboratory.[1–3] Maintenance of youthful morphology, reduced accumulation of oxidized and glycoxidized abnormal proteins, increased ability to decompose H_2O_2, increased activity of the proteosome, reduced accumulation of lipofuscin, and increased resistance to various stresses are some of the main hormetic effects observed.[1–3] Although the beneficial cellular and biochemical effects of RMHS on human cells have been well documented, it is still not clear how cells sense stress and which signaling mechanisms are involved.

The mitogen-activated protein kinases (MAP kinases) are major components of the pathways controlling embryogenesis, cell differentiation, cell proliferation, and cell death. MAP kinases are a family of serine and threonine kinases,

Address for correspondence: Dr. Suresh I.S. Rattan, Department of Molecular Biology, University of Aarhus, Gustav Wieds Vej 10C, DK8000 Aarhus−C, Denmark. Voice: +45 8942 5034; fax: +45 8612 3178.
e-mail: elisernielsen@hotmail.com

Ann. N.Y. Acad. Sci. 1067: 343–348 (2006).
doi: 10.1196/annals.1354.048

whose function and regulation have been highly conserved throughout evolution in organisms as simple as the unicellular brewer's yeast to complex organisms as humans.[4,5] The MAP kinases can be activated by many different stimuli, such as growth factors, cellular stress, cytokines, hormones, and cell–cell adherence. Most of the substrates for MAP kinases are transcription factors, but MAP kinases are also able to phosphorylate protein kinases, phospholipases, and cytoskeleton-associated proteins, and thereby control gene expression.[4,5] Here we report the effects of RMHS at 41°C and 42°C on the levels of the MAP kinases JNK1, JNK2, p38, and ERK2 in serially passaged human skin fibroblasts.

MATERIALS AND METHODS

Normal adult human mammary skin fibroblast cell line designated ASF2 was established from explant skin biopsy from a healthy young donor (aged 28 years). Cells were cultured in complete medium (Dulbecco's modified Eagle's medium, DMEM, [BioWhittaker; now Cambrex, Copenhagen, Denmark] containing 10% (v/v) fetal calf serum [Hyclone, Bie & Berntsen Copenhagen, Denmark], 1% (v/v) glutamine [BioWhittaker], and 1% (v/v) penicillin/streptomycin [BioWhittaker]) at 37°C at 5% CO_2 and 95% humidity. In this experiment we used a control cell line at 37°C, a RMHS at 41°C cell line, and a RMHS at 42°C cell line. The RMHS cell lines were exposed to 1-h heat shock (HS) at the indicated temperature twice a week throughout the life span of the cells.[1]

Proteins were harvested immediately after the last HS in cold lysis buffer, and soluble protein extracts were prepared by centrifuging the samples for 15 min at 13,000 rpm and collecting the supernatant. Proteins were separated by SDS-PAGE followed by Western blotting (10 μg protein for total MAP kinase assays and 30 μg protein for phosphorylated MAP kinase assays), and analyzed after incubation with following antibodies: ERK2 (sc-154), JNK1 (sc-571), JNK2 (sc-572), p38 (sc-535), p-ERK (sc-7383), p-JNK (sc-6254), all purchased from Santa Cruz Biotechnology.

RESULTS AND DISCUSSION

FIGURE 1 shows the longevity curves of the three cell lines, which grew at a similar rate until cumulative population-doubling level (CPDL) 15, by which time they had received approximately 20 rounds of RMHS, after which a difference in the growth curves of the three lines could be seen. The control line at 37°C slowed down earlier as compared with the other two lines, and the RMHS 41°C cell line grew faster than the RMHS 42°C cell line. 41°C-treated cells reached a CPDL of 30 followed by the other two lines

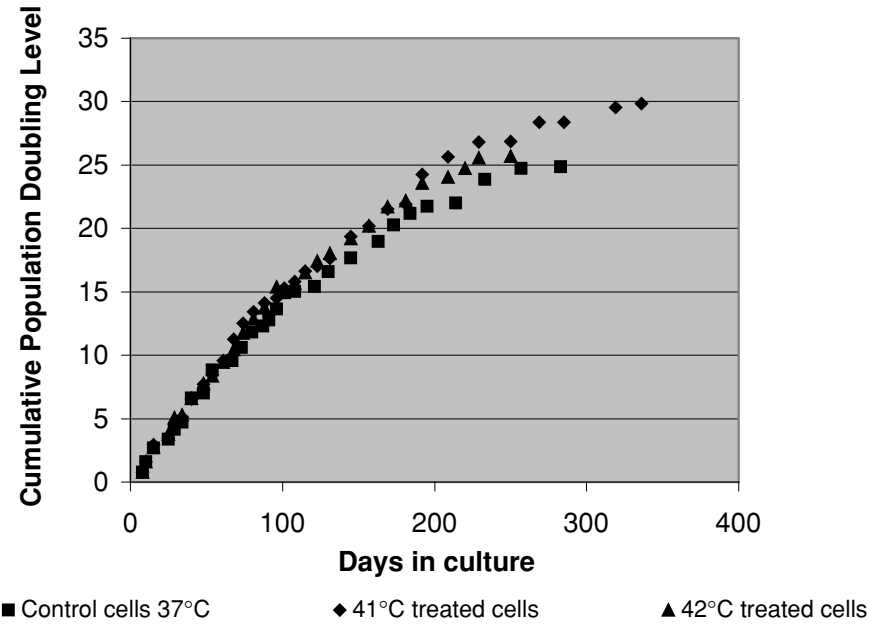

FIGURE 1. Longevity curves of the three cell lines with or without repeated mild heat shock.

(CPDL = 25). Thus there was 20% increase in proliferative life span of human skin fibroblasts exposed to RMHS at 41°C.

FIGURE 2 shows age-related changes in the morphology of the cells, which became larger, flattened, and irregular with serial passaging and had a typical senescent cell phenotype. RMHS appears to slow down the onset of age-related enlargement and irregularization. Of all the three lines, the RMHS 41°C cell line best maintained a relatively youthful morphology in terms of small size, spindle-shaped cells, fewer multinucleated cells, and a more parallel positioning of the cells. The RMHS 42°C cell line also appeared to prevent age-related changes, but the effect was smaller than at 41°C. On the basis of the longevity curves, morphology, and senescence-associated β-galactosidase activity (data not shown), we conclude that the RMHS 41°C cell line aged more slowly than the other two lines.

FIGURE 3 shows the levels of the MAP kinases JNK1, JNK2, p38, and ERK2. The data are presented for three age groups: early-passage young (less than 30% life span completed), middle-aged (45–70% life span completed), and late-passage senescent (more than 80% life span completed) cells. There are no results for ERK1, because the antibody used in this experiment did not recognize ERK1 sufficiently for reliable quantification.

The amount of the stress-activated protein kinases JNK1, JNK2, and p38 increased with age in the control cells. The most dramatic increase was in

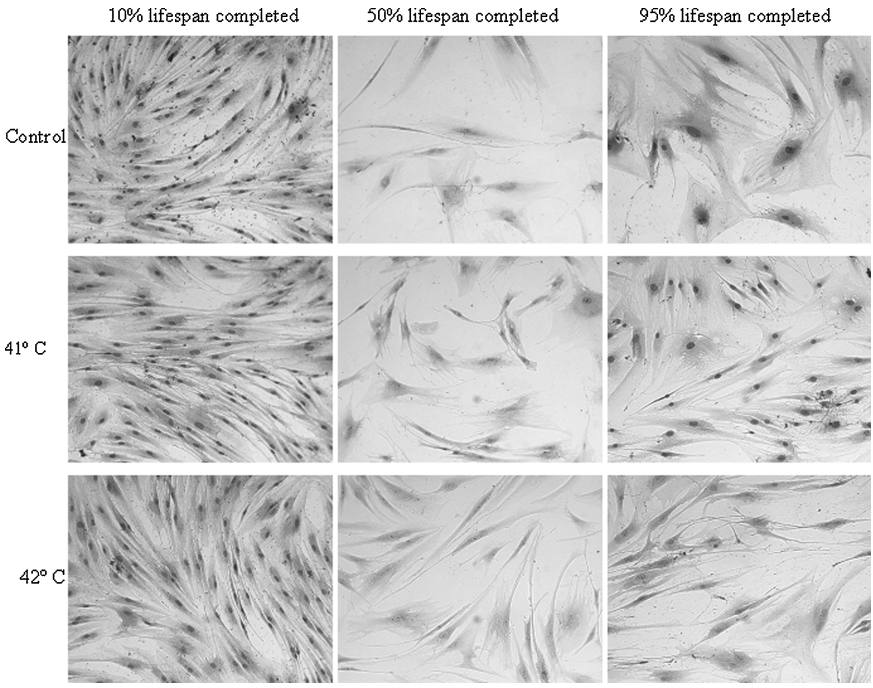

FIGURE 2. Changes in the morphology of serially passaged human skin fibroblasts with or without repeated mild heat shock.

JNK2, where it was more than four times higher in senescent cells than in young cells. In comparison, the levels of JNK1 and p38 increased by 45–70% in senescent cells. In RMHS-treated cells the levels of JNK1, JNK2, and p38 were higher in the middle-aged cells than in the controls. In late-passage senescent cells, the levels of JNK1 and JNK2 were almost similar in all three cell lines, but that of p38 was significantly reduced. In the case of ERK2 the amount was approximately 20% lower in middle-aged and senescent cells as compared to the young cells for all three cell lines (FIG. 3).

Higher levels of MAP kinases in the middle-aged and senescent cells could indicate that the upregulation of the kinases with age may be an adaptive response for cell survival. The increase seen with age can be caused by the constitutive stress coming from the accumulation of damaged proteins. RMHS may therefore act as a signal for enhancing this adaptive response. It will be interesting to find out whether downstream processes affected by MAP kinases are affected in a similar way in normal and RMHS-stressed cells.

We have also determined the basal levels of the phosphorylated (active) form of both ERK1 and ERK2 in serially passaged control cells, which were found to be 4–10 times higher in the middle-aged and senescent cells as compared to the young cells (data not shown). However, the basal activation level of JNK1

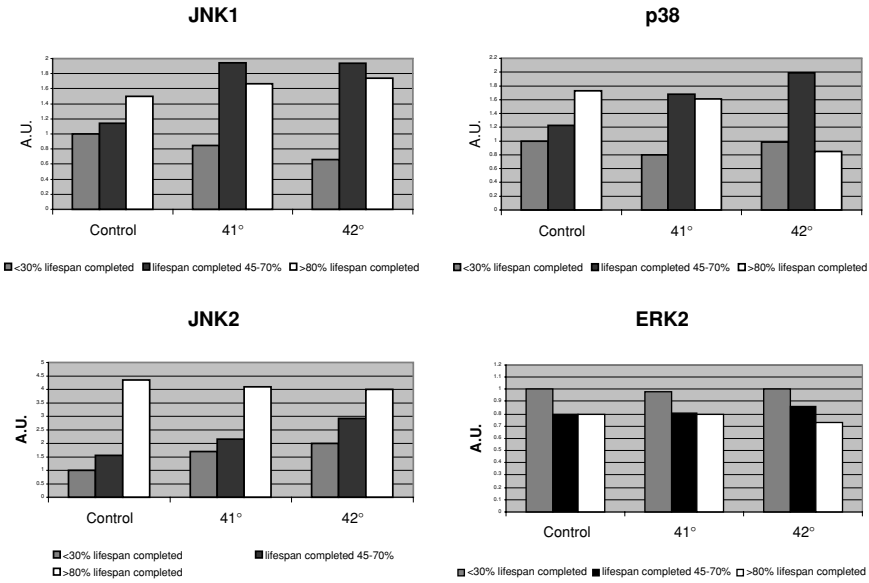

FIGURE 3. Relative amounts (arbitrary units) of MAP kinase proteins in serially passaged human skin fibroblasts with or without repeated mild heat shock.

decreased by 50% in the late-passage cells. This decrease in activated JNK could be due to an increase in Hsp70 levels,[6] which is known to suppress JNK activation. It is believed to be the balance between phosphorylated ERK on one side and phosphorylated JNK and p38 on the other that determines whether the cell will proliferate, differentiate, or undergo apoptosis. The age-related changes observed could indicate that this balance shifts with age, so the senescent cells need a stronger proliferation signal from ERK compared to the apoptotic signals from JNK and p38 than they do at young age.

ACKNOWLEDGMENTS

We thank Anne Gylling, Helle Jakobsen, and Gunhild Siboska for their help and critical discussions. The Laboratory of Cellular Ageing is supported by research grants from the Danish Research Councils, Carlsberg Fund, Senetek PLC, and EU's Biomed Health Programmes.

REFERENCES

1. VERBEKE, P., B.F.C. CLARK & S.I.S. RATTAN. 2001. Reduced levels of oxidized and glycoxidized proteins in human fibroblasts exposed to repeated mild heat shock during serial passaging in vitro. Free Radic. Biol. Med. **31:** 1593–1602.

2. RATTAN, S.I.S. 2004. Aging intervention, prevention, and therapy through hormesis. J. Gerontol. A. Biol. Sci. Med. Sci. **59:** B705–B709.
3. RATTAN, S.I.S. *et al*. 2004. Slowing down aging from within: mechanistic aspects of anti-aging hormetic effects of mild heat stress on human cells. Acta. Biochim. Pol. **51:** 481–492.
4. WIDMANN, C. *et al*. 1999. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol. Rev. **79:** 143–180.
5. PEARSON, G. *et al*. 2001. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr. Rev. **22:** 153–183.
6. FONAGER, J. *et al*. 2002. Mild stress-induced stimulation of heat-shock protein synthesis and improved functional ability of human fibroblasts undergoing aging in vitro. Exp. Gerontol. **37:** 1223–1228.