FEBS Letters 575 (2004) 30–34 FEBS 28777

Involvement of IL-1 family proteins in p38 linked cellular senescence of mouse embryonic fibroblasts

Natsuko Uekawa[a], Akihiko Nishikimi[a], Ken-ichi Isobe[b], Yoichiro Iwakura[c], Mitsuo Maruyama[a,*]

[a]*Laboratory of Experimental Animal Model Research, National institute for longevity sciences, National Center for Geriatrics and Gerontology, 36-3, Gengo, Morioka-Cho, Obu-city, Aichi 474-8522, Japan*
[b]*Department of Mechanism of Aging, National institute for longevity sciences, National Center for Geriatrics and Gerontology, 36-3, Gengo, Morioka-Cho, Obu-city, Aichi 474-8522, Japan*
[c]*Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan*

Received 3 August 2004; revised 12 August 2004; accepted 15 August 2004

Available online 28 August 2004

Edited by Varda Rotter

Abstract Senescence of mammalian cultured cells is essentially organized by a machinery of cell division and cellular stresses induced by various extracellular stimuli. Here, we show that in mouse embryonic fibroblasts (MEFs) culture in vitro, expression of an inflammatory cytokine, interleukin-1β (IL-1β) and its antagonist, IL-1 receptor antagonist (IL-1Ra) are induced by senescence. The kinetics of IL-1β-expression was similar to that of p38 activation during MEFs culture. We also found a distinguishable accelerated senescence in cell growth in IL-1Ra deficient MEFs culture. Our results suggest that IL-1β signaling pathway is involved in activation of p38 linked cellular senescence.

Keywords: Activated p38; Cellular senescence; Interleukin-1β; IL-1 receptor antagonist; Mouse embryonic fibroblast

1. Introduction

After serial cultivation, primary mammalian cells eventually reach a non-dividing state defined as senescence [1]. Senescence in human cells has been widely known as a result driven by the shortening of telomeres (replicative senescence) [2]. In contradistinction, it is also recognized that senescence is induced by telomere-independent manners, as senescence is known to be induced by inadequate culture conditions (culture shock) in mouse embryonic fibroblasts (MEFs) [1]. Thus, cellular senescence seems to be a mechanism for organism to eliminate damaged cells and dodge the risk of developing cancer in both murine and human systems [3]. However, the molecular details of senescence remain mostly unknown.

Mitogen-activated kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases and regulate diverse cellular responses. Activation of MAPKs occurs through phosphorylation by MAPK kinases and this cascade is induced by various extracellular stimuli such as growth factors, inflammatory cytokines and oxdative stress [4]. Although it has been reported that p38, one of the MAPKs family, plays an important role in cellular senescence [5], the role of the p38 signaling cascade, especially which pathway is involved in the activation of p38 during cellular senescence, is still controversial.

In this study, we have attempted to identify the responsible genes for regulating cellular senescence of MEFs by suppression subtractive hybridization [6,7]. We found here that pro-inflammatory cytokines, interleukin-1β (IL-1β) and IL-1 receptor antagonist (IL-1Ra), were upregulated in senescent MEFs. Especially, we provide an evidence that senescence associated IL-1β expression culminates following IL-1Ra expression, but in parallel with p38 activation. Furthermore, we demonstrate that MEFs prepared from IL-1Ra deficient (IL-1Ra$^{-/-}$) mice [8] show an accelerated cell growth decline in conjunction with p38 activation, suggesting that IL-1 signal is involved in p38 activation which plays a causative role of cellular senescence. Our results are discussed in light of hitherto unknown function of IL-1 signaling pathway in cellular senescence.

2. Materials and methods

2.1. Preparation of MEFs and cell culture

C57BL/6 and IL-1Ra$^{-/-}$ (C57BL/6) mice [8] were used to prepare MEFs as follows: E13.5 embryos were minced and trypsinized. The resulting dispersed cells were cultured for 2 days, then cells were frozen in aliquots and considered passage 0 (P0). Serial 3T3 cultivation was done with slight modification [9]. Briefly, 1×10^6 cells were plated in 10 cm dish, 3 days later the cells were passaged in the same scale (1×10^6 cells). When the proliferation declined, cells were subcultured at a splitting ratio of 1:3–1:1 every 3 days. Unless otherwise stated, MEFs were maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma, St. Louis, MO, USA) with 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA) and incubated at 37 °C in 5% CO_2. For growth curves, population doublings (PD) were estimated at each passage using the equation as described previously [10]. For synchronization of the cell cycle at the G1/S boundary, MEFs were washed three times with PBS and incubated in DMEM containing 0.1% FBS for 72 h before preparation of total RNA.

* Corresponding author. Fax: +81-562-46-3157.
E-mail address: michan@nils.go.jp (M. Maruyama).

Abbreviations: MEFs, mouse embryonic fibroblasts; MAPKs, mitogen-activated kinases; IL-1, interleukin-1; IL-1Ra, IL-1 receptor antagonist; IL-1Ra$^{-/-}$, IL-1Ra deficient; PD, population doublings; SA-β-gal, senescence associated β-galactosidase; sIL-1Ra, secreted type IL-1Ra; icIL-1Ra, intracellular IL-1Ra; IL-1RAcP, IL-1R accessory protein

0014-5793/$22.00
doi:10.1016/j.febslet.2004.08.033

2.2. Senescence associated β-galactosidase activity

For senescence associated β-galactosidase (SA-β-gal) activity, approximately 10^5 cells were placed in 3.5 cm dish and cultured for 3 days, then stained with SA-β-gal staining kit in accordance with the manufacturer's instructions (Cell Signaling Technology, Beverly, MA, USA).

2.3. BrdU incorporation

Subconfluent MEFs were metabolically labeled with 10 μg/ml BrdU for 4 h, trypsinized cells were fixed and stained with FITC-conjugated anti-BrdU antibody and 7-AAD in accordance with the manufacturer's instructions (Becton Dickinson, Mountain View, CA, USA).

2.4. Subtractive suppression hybridization

Total RNA was prepared from young (day 4) and senescent (day 16) MEFs using TRIZOL reagent (Invitrogen) and purified poly $(A)^+$ RNA using Nucleo TRAP mRNA kit (Macherey-Nagel, Duren, Germany). 1 μg of total RNA was used for reverse transcription (RT) experiment with SMART™ PCR cDNA Synthesis Kit (BD Clontech, Palo Alto, CA, USA). PCR-Select cDNA subtraction kit was used for subtractive hybridization in accordance with the manufacturer's instructions (BD Clontech). Suppression PCR was performed to prepare two cDNA pools, enriched for genes accumulated in senescent cells (forward-subtracted) and in young cells (reverse-subtracted). Forward-subtracted cDNA pool was screened by colony array using PCR-Select Differential Screening Kit (BD Clonthech) and forward/reverse-subtracted and non-subtracted young and senescent cDNA pools as probes. The clones hybridized with only forward-subtracted probe or both probes of forward-subtracted and non-subtracted senescent cDNA pools were isolated and analyzed their sequences with GenBank database.

2.5. Quantitative real-time RT-PCR analysis

RT experiments were done with SuperScript II (Invitrogen) and quantitative real-time RT-PCR was performed with Smart Cycler (Cepheid, Sunnyvale, CA, USA) and SYBR Green I system (Eurogentec, Seraing, Belgium). Relative mRNA expression levels of each PD were obtained by the following formula: Relative mRNA level $= 2^{(\mathrm{Rt}-\mathrm{Et})\text{ of various day in culture}}/2^{(\mathrm{Rt}-\mathrm{Et})\text{ of reference}}$. The threshold cycle of β-actin and each experimental gene is represented as Rt and Et, respectively. As the reference, the threshold cycle at day 4 was used except IL-1β amplification (day 7).

2.6. Western blot analysis

After treatment with 10 % trichloroacetic acid for 15 min, whole cell lysates were prepared with the sample buffer described previously [11]. For the exogenous recombinant cytokine supplement experiments, MEFs (day 2–5; 1×10^5 cells/3.5 cm dish) were treated with rIL-1β (R&D Systems, Minneapolis, MN, USA) at concentrations as described in the text. Lysates were prepared in the ice cold lysis buffer described previously [12]. Primary antibodies were as follows: p38, pp38 (Cell Signaling Technology); mouse IL-1Ra (M-20), mouse p16 (M-156) (Santa Cruz Biotechnology, Santa Cruz, CA, USA); and β-actin (Sigma). For secondary antibodies, we used Horseradish peroxidase-conjugated donkey anti rabbit and sheep anti mouse (Amersham, Little Chalfont, UK) and rabbit anti goat (Southern Biotechnology, Birmingham, AL, USA) antibodies.

2.7. Primers

For real-time RT-PCR, the following primers were used: β-actin, 5-AAG TGT GAC GTT GAC ATC CGT-3 (sense) and 5-GCA GCT CAG TAA CAG TCC GC-3 (antisense); cytochrome *b*, 5-ATT ATC GCG GCC CTA GCA AT-3 (sense) and 5-GGG CGG AAT ATT AGG CTT CG-3 (antisense); amyloid-β precursor, 5-TGG CCC TCG AGA ATT ACA TC-3 (sense) and 5-ACC GCA GGG ACA TTG TAG AG-3 (antisense); fibronectin 1, 5-TGG AGC CAT GTT CTC AGC TT-3 (sense) and 5-AAG CAG AGG TGT CTG GGT GA-3 (antisense); intracellular IL-1Ra (icIL-1Ra), 5-CTG GGA AGG TCT GTG CCA TA-3 (sense) and 5-CCA TGG GTG AGC TAA ACA GG-3 (antisense); secreted type IL-1Ra (sIL-1Ra), 5-AGC ACA GGC TGG TGA ATG AC-3 (sense) and 5-GTA GGG TCC CCA GCA GAT TT-3 (antisense); IL-1α, 5-TTC TGC ATG GCA TTC TTA GG-3 (sense) and 5-GGT GCA CAG TGA GAT TGG TC-3 (antisense); IL-1β, 5-GCA GAG TTC CCC AAC TGG TA-3 (sense) and 5-GGT TTC TTG TGA CCC TGA GC-3 (antisense); IL-1R type1, 5-CCA CCA GAC AAG GAG-3 (sense) and 5-AGG CCT GGG TCC AGT AGA AT-3 (antisense); IL-1R type2, 5-CTG GAA GGT GAA CCT GTG GT-3 (sense) and 5-GGC GCC CTT ATA CCA CTG TA-3 (antisense).

3. Results

3.1. Senescence-associated gene expression in MEFs

We have cultured MEFs derived from C57BL/6 mice as described in materials and methods. As expected, the cells grew vigorously for approximately the initial 10 days, then their proliferation tended to decline (Fig. 1A). Around 2 weeks later, the cultures senesced and represented little change in cell number for a few weeks. Eventually, proliferation resumed because of appearance and outgrowth of immortal cell population (over 45 days). To further characterize a reduction of cell growth in MEFs culture by the capacity for DNA synthesis, BrdU incorporation was measured at 2, 11–23 and 50 days in culture. After 4 h incubation with BrdU, the percentage of BrdU positive MEFs both in 'young' and 'immortal' cells represented around 45%, despite notable decrease below 15% incorporation shown in senescent MEFs (Fig. 1B). After about 10–20 days in culture, the morphological change was confirmed by enlarged and flattened shape and expression of SA-β-gal, which is available biomarker for senescent cells (Fig. 1C and data not shown). On the basis of the observation above, we considered the MEFs between day 13 and 30 as senescent. In order to identify the genes upregulated in senescence, total RNA were prepared from young (day 4) and senescent (day 16) MEFs and subjected to cDNA subtraction. To eliminate a consideration of the genes involved in cell division, MEFs were synchronized with their cell cycle to G1 phase under serum starvation for 72 h before harvest. We obtained 34 candidate clones involving the previously reported senescence associated genes, such as fibronectin [13], amyloid-β precursor [14] and cytochrome *b* [15] (Fig. 1D) as well as genes which were found in only expressed sequence-tagged database, suggesting that our system did function well. Among them, we noted IL-1Ra gene because its expression determined by quantitative real-time RT-PCR was dramatically upregulated in senescent MEFs (about 14-fold. Fig. 1D.)

3.2. Kinetics of IL-1 family gene expression and p38 activation in MEFs senescence

Two forms of protein sIL-1Ra and icIL-1Ra type, has been reported in mice [16]. Therefore, we next examined each mRNA expression of sIL-1Ra or icIL-1Ra by quantitative real-time RT-PCR using the unique primers for each IL-1Ra, since the identified cDNA fragment was common to both forms of IL-1Ra (Fig. 1D). As shown in Fig. 2A and B, we found that sIL-1Ra and icIL-1Ra similarly increased more than 20-fold by day 10, prior to senescent phase. Because IL-1Ra is one of the IL-1 family member, we analyzed the gene expression profiles of other IL-1 family agonists and their receptors in various culture days. About agonists, although IL-1α mRNA showed only slight increase (less than 2-fold) in senescent MEFs, IL-1β mRNA increased specifically through the senescent phase (over 5-fold) and declined in immortal MEFs (Fig. 2C and D). On the other hand, the expression of functional receptor (IL-1R1) and 'decoy receptor' (IL-1R2) also increased on day 10 (Fig. 2E and G) coincidentally with

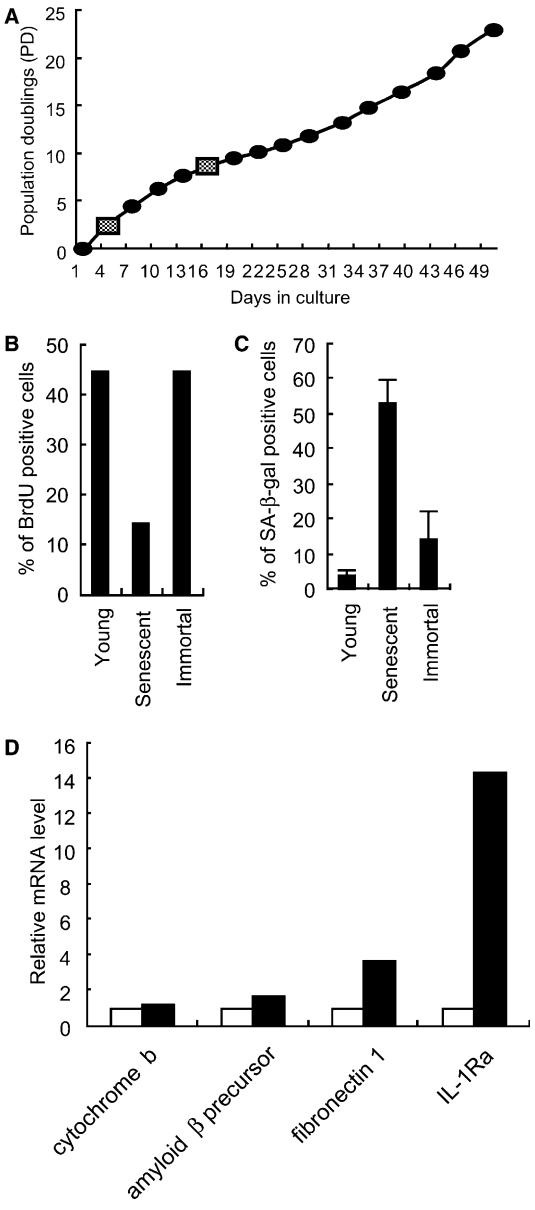

Fig. 1. Characterization of cellular senescence in MEFs and senescence-associated genes. (A) Growth curve of MEFs. Cells were cultivated as described in Section 2. The squares show the point at which total RNA were prepared for suppression subtractive hybridization. (B) Proliferation index in MEFs. Percentage of cells in S phase was measured by BrdU incorporation. Young: day 2, senescent: day 11–23, and immortal: day 50. (C) Comparison of SA-β-gal activity. Percentages of SA-β-gal activity positive cells in young (day 1–4), senescent (day 10–20) and immortal (day 30–40) MEFs are shown. Means values and S.D. were derived from three experiments. (D) Expression of the genes identified by cDNA subtraction screening. Each bar represents the relative fold increase of mRNA level in G1-synchronized MEFs at day 16 (black) compared with day 4 (white) after normalization to β-actin.

IL-1Ra. Interestingly, these mRNA levels dropped on day 13 once and only both IL-1Ra levels were upregulated again through senescence phase (day 13–34). Concerning another essential component for IL-1 signaling, IL-1R accessory protein (IL-1RAcP), an ubiquitous expression was detected in early phase of senescence and precise increase was observed following IL-1β expression (Fig. 2F).

Because it has been reported that IL-1 can activate p38 MAPK [17], which plays a causative role in senescence [5], we next examined the kinetics of p38 activation in MEFs (Fig. 3). The accumulation of phosphorylated p38 (pp38) protein was increased drastically until day 21 and this level was continued during senescence paralleled with that of p16 cell cycle inhibitor. Combining the kinetics of IL-1β mRNA expression with this result, it is strongly suggested that IL-1β functions actively in concert with pp38 in MEFs senescence. On the other hand, pp38 level was low at early days in culture when IL-1Ra was increasing in mRNA and the following protein expression (day 9–15; Figs. 2A, B and 3). This suggests that IL-1Ra negatively regulates p38 activation and senescence until IL-1β surpass IL-1Ra in expression level.

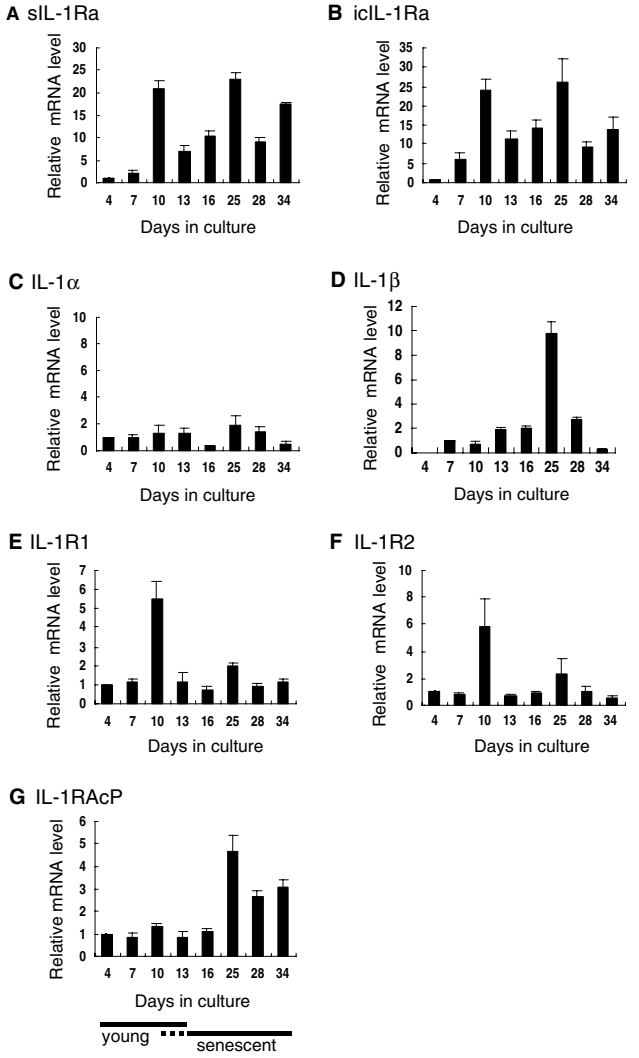

Fig. 2. mRNA expression of IL-1 family and its receptors. Each bar represents the relative fold increase of mRNA level in various MEFs indicated days in cultures compared with day 4 (day 7 for IL-1β) after normalization to β-actin. Values represent means ± S.D. of four data sets. (A) sIL-1Ra, (B) icIL-1Ra, (C) IL-1α, (D) IL-1β, (E) IL-1R1, (F) IL-1RAcP, (G) IL-1R2. ND, not detected. Typical young and senescent phases in culture days are described under panel (G).

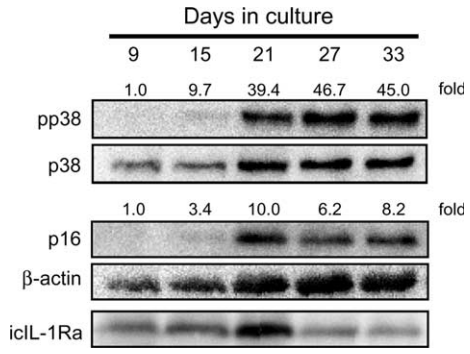

Fig. 3. IL-1 related proteins increased with cellular senescence in C57BL/6 MEFs. Immunoblot analysis of pp38, p38, p16 and icIL-1Ra expression was carried out with cell extracts from various MEFs indicated days in cultures. The protein levels normalized to those of β-actin (p16) or p38 (pp38) are shown as folded values relative to that of MEFs at day 9. Day 9 and day 15–33 correspond to young and senescent phase, respectively.

3.3. *IL-1Ra$^{-/-}$ MEFs represent hastened senescence in vitro culture*

In view of the physiological effect by IL-1 family in cellular senescence, we next cultured MEFs prepared from IL-1Ra$^{-/-}$ mice. All in all, IL-1Ra$^{-/-}$ MEFs displayed similar morphological changes to wild-type (data not shown), however, we noticed that the primary proliferation in IL-1Ra$^{-/-}$ MEFs declined slightly earlier than wild-type control (Fig. 4A). This difference was manifested and statistically significant

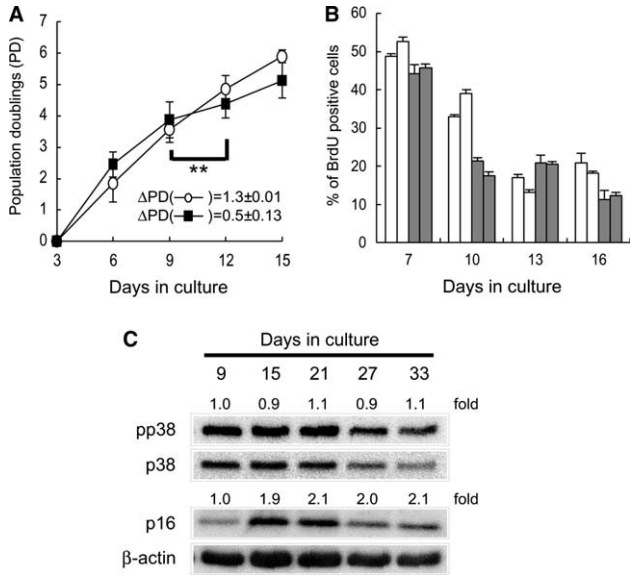

Fig. 4. Cellular senescence is hastened in IL-1Ra$^{-/-}$ MEFs. (A) Growth curves of MEFs from C57BL/6 (wt) (open circle) or IL-1Ra$^{-/-}$ (black square) mice. The data represent means ± S.D. of three independent experiments. Statistical significance for means of PD per 3 days was determined by ANOVA. $^{**}P < 0.01$. (B) The proliferation index in early senescent MEFs from wt (open bar) or IL-1Ra$^{-/-}$ (dotted bar) mice was determined by BrdU incorporation. The data shown are two sets of results representing means ± S.D. of three independent experiments. (C) Immunoblot analyses of p38, pp38 and p16 expression were carried out in various IL-1Ra$^{-/-}$ MEFs indicated days in cultures. Each protein level is normalized to p38 (for pp38) or β-actin (for p16) and described as relative value to IL-1Ra$^{-/-}$ MEFs at day 9.

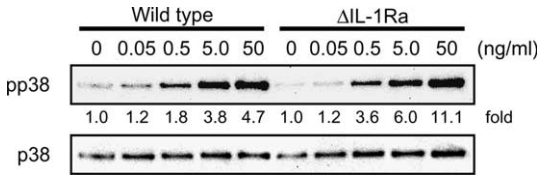

Fig. 5. IL-1β induced activation of p38 in MEFs. Immunoblot analysis of pp38 in C57BL/6 (wt) and IL-1Ra$^{-/-}$ MEFs (~day 5) after incubation with increasing amounts of rIL-1β described for 5 min. Protein level of pp38 is normalized to p38 and shown as relative value.

($P < 0.01$) in day 9–12. To confirm this reproducible reduction in cell proliferation, BrdU incorporation was measured in IL-1Ra$^{-/-}$ MEFs culture. As shown in Fig. 4B, BrdU incorporation of IL-1Ra$^{-/-}$ MEFs was obviously lower than wild-type in the early phase of senescence, day 10, although the difference was not so distinguishable in the following senescent phase. The young (day 5) wild-type and IL-1Ra$^{-/-}$ MEFs showed comparable population of spontaneously dead cells in annexin-V assay (data not shown), therefore we concluded that this reduced proliferation of IL-1Ra$^{-/-}$ MEFs was not the result of increased cell death.

To gain more insights into the molecular pathway with the MEFs senescence, we next examined the kinetics of p38 activation in IL-1Ra$^{-/-}$ MEFs. As contrasted with wild-type MEFs in Fig. 3, both a striking p38 activation and p16 accumulation were observed at earlier days in IL-1Ra$^{-/-}$ MEFs culture (Fig. 4C). These results suggest that under the absence of IL-1Ra, IL-1β can smoothly activate the downstream p38 and subsequently promote a relative swift cellular senescence in MEFs. To confirm this, we investigated whether p38 is activated more easily without IL-1Ra in MEFs. As shown in Fig. 5, significant phosphorylation of p38 was confirmed in dose dependent manner only 5 min after supplement of rIL-1β. It is worth to note that IL-1Ra$^{-/-}$ MEFs represent about 2-fold sensitivity to rIL-1β at the 0.5–50 ng/ml of dose. Thus, we concluded that IL-1 signaling was involved in p38 activation during senescence and IL-1Ra have somehow negative function against senescence through inhibition of IL-1 signaling in MEFs.

4. Discussion

Recently, it has been reported that p38 MAPK plays an important role in cellular senescence, but which pathway is involved in p38 activation in senescent cells is still unknown. In this study, we focused on inflammatory cytokine IL-1β and IL-1Ra, because mRNA expression level of these genes increased during senescence of MEFs (Fig. 2A, B and D). To date, although it has been reported that IL-1α or even both IL-1α/β expression elevated in senescence of human endothelial cells [18] or fibroblast cells [19], they simply discussed about IL-1-induced gene expression during senescence. Thus, we examined the function of IL-1β signaling including IL-1Ra for cellular senescence mediated by activation of p38 MAP kinase.

In the present study, we showed that the kinetics of IL-1β expression was correlated with the kinetics of p38 activation in MEFs senescence (Figs. 2 and 3) and in IL-1Ra$^{-/-}$ MEFs, p38 activation and growth reduction were likely to be induced

earlier than wild-type MEFs (Fig. 4). These results suggest that IL-1 signal is involved in activation of p38 during senescence. The coupled age-related increase in IL-1β concentration and p38 activation has been recently reported in neuronal tissue from rat brain [20,21]. This information surely lends further support to notion that IL-1β signaling with p38 activation is involved in MEFs senescence in vitro as well as in the aged rat brain.

IL-1Ra expression was increasing during early days in culture and at that phase p38 activation was suppressed. But at day 21, p38 was activated intensively though IL-1Ra expression level was high (Figs. 2 and 3). It is reported that 100-fold or greater levels of IL-1Ra over IL-1 are necessary to functionally inhibit the biologic effects of IL-1 on target cells [22]. So we concluded that IL-1Ra was overcome by IL-1β on day 21 (Fig. 2D).

In this paper, our results indicate that IL-1Ra function as a negative regulator to senescence through inhibition of IL-1 signaling. Alternatively, it has been recently reported that IL-1Ra allelic polymorphisms affect replicative lifespan of human endothelial cells [23] and icIL-1Ra expression preserved young phenotype in endometrial stromal fibroblasts [24]. And there is another report that IL-1Ra$^{-/-}$ mice represented early mortality compared to wild-type mice [25]. Thus, we strongly suggest here that preventive function of IL-1Ra in IL-1 signaling involved senescence must be considered indistinguishable in terms of both in vitro and in vivo.

It is also showed that imbalance between IL-1β and IL-1Ra is a trigger in many diseases, especially those suffered by elderly people such as cancer and rheumatoid arthritis [26]. Hence, the physiological role of IL-1 family is seemed to be critical for the health in aged people. In addition, there is another report that pp38 was increased in the Alzheimer's disease brain relative to age matched normal human brain [27], and we observed that mRNA expression of both types of IL-1Ra was upregulated in senescent human fibroblasts cells, MRC-5 (N.U. and M.M., unpublished data). Therefore, in view of clinical point, to reveal the physiological role of IL-1β and IL-1Ra in senescent MEFs will be an inevitable issue for providing useful therapeutic information of these diseases.

Acknowledgements: We thank Drs. A. Matsuura, N. Motoyama and T. Miyazaki for their invaluable discussion and advice. We also appreciate Drs. F. Ishikawa and L. Pao for critical comments on the manuscript. This work was supported in part by Research Grant for Longevity Sciences (16A-2) from the Ministry of Health, Labore and Welfare of Japan. A.N. is a research fellow of the Japan Society for the Promotion of Science.

References

[1] Sherr, C.J. and DePinho, R.A. (2000) Cell 102, 407–410.
[2] Campisi, J. (2001) Exp. Gerontol. 36, 607–618.
[3] Campisi, J. (2003) Exp. Gerontol. 38, 5–11.
[4] Chang, L. and Karin, M. (2001) Nature 410, 37–40.
[5] Iwasa, H., Han, J. and Ishikawa, F. (2003) Genes Cells 8, 131–144.
[6] Ito, T., Kito, K., Adati, N., Mitsui, Y., Hagiwara, H. and Sakaki, Y. (1994) FEBS Lett. 351, 231–236.
[7] Ito, T. and Sakaki, Y. (1999) Methods Enzymol. 303, 298–309.
[8] Matsuki, T., Horai, R., Sudo, K. and Iwakura, Y. (2003) J. Exp. Med. 198, 877–888.
[9] Todaro, G.J. and Green, H. (1963) J. Cell Biol. 17, 299–313.
[10] Pantoja, C. and Serrano, M. (1999) Oncogene 18, 4974–4982.
[11] Laemmli, U.K. (1970) Nature 227, 680–685.
[12] Adachi, Y., Pavlakis, G.N. and Copeland, T.D. (1994) J. Biol. Chem. 269, 2258–2262.
[13] Choi, A.M., Olsen, D.R., Cook, K.G., Deamond, S.F., Uitto, J. and Bruce, S.A. (1992) J. Cell Physiol. 151, 147–155.
[14] Adler, M.J., Coronel, C., Shelton, E., Seegmiller, J.E. and Dewji, N.N. (1991) Proc. Natl. Acad. Sci. USA 88, 16–20.
[15] Kodama, S., Yamada, H., Annab, L. and Barrett, J.C. (1995) Exp. Cell Res. 219, 82–86.
[16] Gabay, C., Porter, B., Fantuzzi, G. and Arend, W.P. (1997) J. Immunol. 159, 5905–5913.
[17] Raingeaud, J., Gupta, S., Rogers, J.S., Dickens, M., Han, J., Ulevitch, R.J. and Davis, R.J. (1995) J. Biol. Chem. 270, 7420–7426.
[18] Maier, J.A., Voulalas, P., Roeder, D. and Maciag, T. (1990) Science 249, 1570–1574.
[19] Kumar, S., Millis, A.J. and Baglioni, C. (1992) Proc. Natl. Acad. Sci. USA 89, 4683–4687.
[20] Martin, D.S., Lonergan, P.E., Boland, B., Fogarty, M.P., Brady, M., Horrobin, D.F., Campbell, V.A. and Lynch, M.A. (2002) J. Biol. Chem. 277, 34239–34246.
[21] Murray, C.A. and Lynch, M.A. (1998) J. Neurosci. 18, 2974–2981.
[22] Arend, W.P., Welgus, H.G., Thompson, R.C. and Eisenberg, S.P. (1990) J. Clin. Invest. 85, 1694–1697.
[23] Dewberry, R., Holden, H., Crossman, D. and Francis, S. (2000) Arterioscler. Thromb. Vasc. Biol. 20, 2394–2400.
[24] Rinehart, C.A., Watson, J.M., Torti, V.R. and Palmieri, D. (1999) Exp. Cell. Res. 248, 599–607.
[25] Irikura, V.M., Lagraoui, M. and Hirsh, D. (2002) J. Immunol. 169, 393–398.
[26] Arend, W.P. (2002) Cytokine Growth Factor Rev. 13, 323–340.
[27] Hensley, K. et al. (1999) J. Neurochem. 72, 2053–2058.