Molecular regulatory mechanisms of osteoclastogenesis through cytoprotective enzymes

Hiroyuki Kanzaki, Fumiaki Shinohara, Itohiya Kanako, Yuuki Yamaguchi, Sari Fukaya, Yutaka Miyamoto, Satoshi Wada, Yoshiki Nakamura

Tohoku University Hospital, Maxillo-Oral Disorders, Japan
Department of orthodontics, School of Dental Medicine, Tsurumi University, Japan
Tohoku University Graduate School of Dentistry, Oral Microbiology, Japan

Abstract

It has been reported that reactive oxygen species (ROS), such as hydrogen peroxide and superoxide, take part in osteoclast differentiation as intra-cellular signaling molecules. The current assumed signaling cascade from RANK to ROS production is RANK, TRAF6, Rac1, and then Nox. The target molecules of ROS in RANKL signaling remain unclear; however, several reports support the theory that NF-κB signaling could be the crucial downstream signaling molecule of RANKL-mediated ROS signaling. Furthermore, ROS exert cytotoxic effects such as peroxidation of lipids and phospholipids and oxidative damage to proteins and DNA. Therefore, cells have several protective mechanisms against oxidative stressors that mainly induce cytoprotective enzymes and ROS scavenging. Three well-known mechanisms regulate cytoprotective enzymes including Nrf2-, FOXO-, and sirtuin-dependent mechanisms. Several reports have indicated a crosslink between FOXO- and sirtuin-dependent regulatory mechanisms. The agonists against the regulatory mechanisms are reported to induce these cytoprotective enzymes successfully. Some of them inhibit osteoclast differentiation and bone destruction via attenuation of intracellular ROS signaling. In this review article, we discuss the above topics and summarize the current information available on the relationship between cytoprotective enzymes and osteoclastogenesis.

1. Introduction

Osteoclasts are multi-nucleated cells that resorb bone tissue and are differentiated from macrophage–monocyte cell lines. Osteoclast differentiation, namely osteoclastogenesis, is strictly regulated by receptor activator of nuclear factor kappa-B ligand (RANKL), an osteoclastogenic signaling cytokine. Reactive oxygen species (ROS), such as hydrogen peroxide and superoxide, work as intracellular signaling molecules following RANKL signaling during osteoclastogenesis. However, apart from their role as intracellular signaling molecules, ROS exert cytotoxic effects such as peroxidation of lipids and phospholipids, and oxidative damage to proteins and DNA. Therefore, cells have several protective mechanisms against these oxidative stressors most of which induce cytoprotective enzymes and ROS scavenging. Taken together, it is thought that cytoprotective mechanisms are attenuated during osteoclastogenesis to intensify intracellular ROS signaling. In this review article, we have summarized the relationship between osteoclastogenesis and the protective mechanisms that work against oxidative stressors.

2. ROS work as intracellular signaling molecules during osteoclastogenesis

RANKL is an essential cytokine in osteoclastogenesis, and various intracellular signaling molecules, such as nuclear factor of activated T-cells (NFAT), mitogen-activated protein kinase (MAPK), tumor necrosis factor receptor-associated factor (TRAF), c-jun N-terminal kinase (JNK), Akt, and ROS have been identified. ROS are interesting molecules because not only do they work as intracellular signaling molecules, but also they increase with age or with the onset of an inflammatory state, which subsequently leads to bone destruction.

*Corresponding author at: Department of Orthodontics, School of Dental Medicine, Tsurumi University, Japan.
E-mail address: kanzaki-h@tsurumi-u.ac.jp (H. Kanzaki).

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in the regulation of osteoclastogenesis from both within the cytoplasm and extracellularly.

It is reported that TRAF6 plays a key linkage role in ROS production by RANKL [39]. We reported that dominant-interfering mutant form of TRAF6, significantly decreased ROS induction, although TRAF6 itself does not directly produce ROS. Rac, a functional downstream molecule and member of the Rho-GTPase subfamily, which is involved in the organization of the cytoskeleton, is a cytosolic component of NADPH oxidase (NOX) complex and responsible for the activation of NOxes [40]. The expression of a dominant-negative mutant of Rac1 blocks ROS production, signifying that Rac1 is responsible for regulating the generation of ROS during osteoclast differentiation [41]. In addition, NOxes have been reported as essential enzymes that produce ROS during osteoclast differentiation [42–44]. Taken together, the current assumed signaling cascade from RANK to ROS production is RANK, TRAF6, Rac1, and then NOX.

The target molecules of ROS in RANKL signaling remain unclear; however, several reports have suggested that MAPK, PI3K, and NF-κB activation are downstream events [45,46]. Additionally, Bharti et al. reported that curcumin, which has ROS-scavenging properties, inhibits RANKL-induced NF-κB activation, which indicates that NF-κB signaling could be the crucial downstream signaling molecule of RANKL-mediated ROS signaling [47]. Current information about the intracellular signaling cascade of RANKL is summarized in Fig. 1.

3. Defense mechanisms against ROS

As mentioned previously, ROS exhibit cytotoxicity [7,8]; therefore, cells have several protective mechanisms against these oxidative stressors that mainly induce cytoprotective enzymes and ROS scavenging. The mechanisms regulating cytoprotective enzymes are summarized in Table 1.

The most renowned regulator of cytoprotective enzymes is transcriptional factor nuclear factor E2-related factor 2 (Nrf2), which controls the gene expression of many cytoprotective enzymes, such as heme oxygenase-1 (HO-1) [13], NAD (P) H: quinone reductase (NQO1) [14], gamma-glutamylcysteine synthetase (GCS) [15], and the auxiliary cellular NADPH regenerating enzyme, glucose 6-phosphate dehydrogenase (G6PD) [16] (Fig. 2); all of these enzymes are ROS scavengers [17–20]. However, kelch-like ECH-associated protein 1 (Keap1) negatively regulates Nrf2-dependent transcription of cytoprotective enzymes by inhibiting nuclear translocation, cytoplasmic ubiquitination, and degradation of Nrf2 [48].

4. Cytoprotective enzymes and osteoclastogenesis

Since ROS operate as intracellular signaling molecules during osteoclastogenesis, a close relationship between osteoclastogenesis and cytoprotective enzymes is to be expected. Indeed, a well-known cytoprotective enzyme, HO-1, is a negative regulator of osteoclastogenesis [67–69]. Relationships between the mechanisms regulating cytoprotective enzymes and osteoclastogenesis have also been reported. Rana et al. reported that loss of Nrf2 accelerates ionizing radiation-induced bone loss in Nrf2 knockout mice [70]. Other groups have reported that Nrf2 negatively regulates osteoclastogenesis through attenuation of RANKL-mediated intracellular ROS signaling by cytoprotective enzymes [71,72]. Furthermore, we previously reported that overexpression of Nrf2 induces the expression of cytoprotective enzymes, attenuates intracellular ROS signaling, and thereby inhibits osteoclastogenesis [71]. Both overexpression of Nrf2 and Nrf2 activation (induction of nuclear translocation) inhibit osteoclastogenesis [6,73,74]. These lines of evidence suggest that Nrf2 activation could be a therapeutic approach towards bone destructive diseases such as rheumatoid arthritis, osteoporosis, and periodontitis.

Another mechanism regulating cytoprotective enzyme FOXO contributes to the control of osteoclastogenesis. Bartell et al. reported that FOXO protein attenuates osteoclastogenesis via augmentation of cytoprotective enzymes [75]. Sirtuins, originally identified as protein deacetylases, have been reported as suppressors of osteoclastogenesis. SIRT1 suppresses osteoclastogenesis by the upregulation of cytoprotective enzymes via FOXO-mediated transcription and subsequent attenuation of intracellular ROS signaling [76]. Lee et al. reported that the overexpression of SIRT6, an NAD (+)-dependent deacetylase, suppresses bone destruction in a collagen-induced arthritis mouse model [77]. These lines of evidence suggest that the key molecule among the mechanisms regulating cytoprotective enzymes (Nrf2, FOXO, and
sirtuin) negatively regulates osteoclastogenesis via attenuation of intracellular ROS signaling (Fig. 5).

5. Regulatory mechanisms of potential pharmacological targets for bone destructive diseases

As discussed above, osteoclasts also possess mechanisms that regulate cytoprotective enzymes, which manage the intracellular ROS levels. Since intracellular ROS play a role in RANKL-mediated osteoclastogenesis, the mechanisms that regulate cytoprotective enzymes negatively control osteoclastogenesis via ROS scavenging mediated by cytoprotective enzymes. In other words, osteoclastogenesis is controlled via interference with the mechanisms regulating cytoprotective enzymes.

Indeed, some papers report that the activation of Nrf2 inhibits osteoclastogenesis [6,73,74]. The pharmacological activation of Nrf2 has been extensively explored in cancer research and chemical detoxification fields, thus potential Nrf2 activators such as sulforaphane [78], epigallocatechin gallate [79], curcumin [80], and N-acetylcysteine [81] are well-documented and known to inhibit bone destruction [72,82–84]. Another regulatory molecule, FOXO, is also a potential therapeutic target for bone destructive diseases. Statins, HMG-CoA reductase inhibitors, induce FOXO phosphorylation [85] and exhibit osteoclastogenesis by ROS scavenging [86]. Regarding sirtuin-mediated regulatory mechanisms, resveratrol, an agonist of SIRT1 [87], inhibits osteoclastogenesis through the attenuation of ROS production [88–90]. Indeed, sirtuin activators such as resveratrol or other synthesized chemicals inhibit bone destruction [91–94]. Some of the chemicals reported to activate cytoprotective enzymes and thereby inhibit bone destruction are summarized in Table 2.

6. Summary and perspective

In this review manuscript, we have summarized recent information about the relationship between osteoclastogenesis and the mechanisms regulating cytoprotective enzymes. Although some parts have been extensively explored, further investigations

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**Table 1** Regulatory mechanisms of cytoprotective enzymes.

| Regulator | Cell type/experimental model | Tested function/findings | References |
|-----------|-----------------------------|--------------------------|------------|
| Nrf2      | L929 fibroblast, mutant Nrf2 expression | Mutant Nrf2 decreased HO-1 | [13]       |
| Rat NQO-1 | gene, promoter assay        | Nrf2 regulated NQO-1     | [14]       |
| Human GCS | gene, promoter assay        | Nrf2 regulated GCS       | [15]       |
| Nrf2 KO   | knockout mice               | Nrf2 KO decreased NQO-1 and GCS | [16]       |
| FOXO      | Breast cancer cells         | FOXO3 regulates MnSOD    | [49]       |
| NQO-1     | gene, promoter assay        | FOXO3 regulates MnSOD    | [49]       |
| GCS       | gene, promoter assay        | FOXO3 regulates MnSOD    | [49]       |
| Nrf2 KO   | knockout mice               | FOXO3 regulates MnSOD    | [49]       |
| Mammalian | cells                       | FOXO3 directly increase MnSOD | [54]       |
| Mammalian | cells                       | FOXO3 directly increase MnSOD | [54]       |
| Mouse NIH3T3 | cells                | FOXO3 directly increase MnSOD | [54]       |

**Fig. 2.** Nrf2-mediated cytoprotective enzymes scavenge ROS. Nrf2 transcriptionally regulates the expressions of HO1, GCS, NQO1, and G6PD. HO1 convert heme into carbon oxide (CO) and bilirubin, and they scavenge ROS. GCS increases intracellular glutathione, which results in ROS scavenging. NQO1 reduces oxyradicals. G6PD increases intracellular NADPH, which augments ROS scavenging.

**Fig. 3.** FOXO-mediated cytoprotective enzymes scavenge ROS. FOXO regulates the expressions of MnSOD (SOD2) and catalase (CAT). MnSOD convert superoxide into H2O2, followed by the conversion into H2O and O2 by CAT.

**Fig. 4.** SIRT-mediated cytoprotective enzymes scavenge ROS. SIRT regulates the expressions of MnSOD (SOD2) and catalase (CAT). MnSOD convert superoxide into H2O2, followed by the conversion into H2O and O2 by CAT.
are necessary to gain a greater understanding. In particular, crosstalk among the mechanisms regulating cytoprotective enzymes and other signaling molecules should be elucidated.

Since some of the agonists that affect the mechanisms regulating cytoprotective enzymes have been reported as inhibitors of bone destruction, these chemicals could be potential drugs for the treatment for bone destructive diseases in the near future.

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References

[1] G.D. Roodman, Advances in bone biology: the osteoclast, Endocr. Rev. 17 (1996) 308–332.

[2] S.L. Teitelbaum, M.M. Tondravi, F.P. Ross, Osteoclasts, macrophages, and the molecular mechanisms of bone resorption, J. Leukoc. Biol. 61 (1997) 381–388.

[3] D.L. Lacey, E. Timms, H.L. Tan, M.J. Kelley, C.R. Dunstan, T. Burgess, R. Elliott, A. Colombero, G. Elliott, S. Scully, H. Hsu, J. Sullivan, N. Hawkins, E. Davy, C. Capparelli, A. Eli, Y.X. Qian, S. Kaufman, I. Sarosi, V. Shalhoub, G. Senaldi, J. Guo, J. Delaney, W.J. Boyle, Antioxidative peptide ligand is a cytokine that regulates osteoclast differentiation and activation, Cell 93 (1998) 165–176.

[4] G. Chen, L.V. Favreau, C.B. Pickett, Transcriptional regulation of the rat NAD(P)H:quinone oxidoreductase-1 promoter by hydrogen peroxide, Biochem. Biophys. Res. Commun. 183 (1992) 1153–1158.

[5] H. Ha, H.B. Kwak, S.W. Lee, H.M. Jin, H.M. Kim, H.H. Kim, Z.H. Lee, Reactive oxygen species mediate RANK signaling in osteoclasts, Exp. Cell Res. 301 (2004) 119–127.

[6] H. Kanzaki, F. Shinohara, M. Kajiya, S. Fukaya, Y. Miyamoto, Y. Nakamura, Nuclear factor-κB induction by protein transduction attenuates osteoclastogenesis, Free Radic. Biol. Med. 77 (2014) 239–248.

[7] H. Esterbauer, R.J. Schaur, H. Zollner, Chemistry and biochemistry of 4-hydroxy-2-nonenal, malonaldehyde and related aldehydes, Free Radic. Biol. Med. 11 (1991) 81–128.

[8] H. Kanzaki, F. Shinohara, M. Kajiya, S. Fukaya, Y. Miyamoto, Y. Nakamura, Nuclear factor-κB induction by protein transduction attenuates osteoclastogenesis, Free Radic. Biol. Med. 77 (2014) 239–248.

[9] T.W. Kessler, N. Wakabayashi, S. Biswal, Cell survival responses to environmental stresses via the Keap1–Nrf2–ARE pathway, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 89–116.

[10] Y. Furukawa-Hibi, Y. Kobayashi, C. Chen, N. Motoyama, FOXO transcription factors in cell-cycle regulation and the response to oxidative stress, Antioxid. Redox Signal 7 (2005) 752–760.

[11] C.P. Hsu, I. Oedewale, R.R. Alcendor, J. Sadoshima, Sirt1 protects the heart from aging and stress, Biol. Chem. 389 (2009) 221–231.

[12] J.M. Mateos, F. Sanchez-Jimenez, Antioxidative enzymes and their implications in pathophysiologic processes, Front. Biosci. 4 (1999) D339–D345.

[13] J. Alam, D. Stewart, C. Touchard, S. Boinapally, A.M. Choi, Cook J.L, Nrf2, a Cap’n’Collar transcription factor, regulates induction of the heme oxygenase-1 gene, J. Biol. Chem. 274 (1999) 26071–26078.

[14] L.V. Favreau, C.B. Pickett, Transcriptional regulation of the rat NAD(P)H:quinone oxidoreductase-1 promoter by hydrogen peroxide, Biochem. Biophys. Res. Commun. 183 (1992) 1153–1158.

[15] J. Guo, J. Delaney, W.J. Boyle, Antioxidative peptide ligand is a cytokine that regulates osteoclast differentiation and activation, Cell 93 (1998) 165–176.

[16] P.C. Hsu, I. Odewale, R.R. Alcendor, J. Sadoshima, Sirt1 protects the heart from aging and stress, Biol. Chem. 389 (2009) 221–231.

[17] C.P. Hsu, I. Odewale, R.R. Alcendor, J. Sadoshima, Sirt1 protects the heart from aging and stress, Biol. Chem. 389 (2009) 221–231.

[18] H. Yasuda, N. Shima, N. Nakagawa, K. Yamaguchi, M. Kinosaki, S. Mochizuki, A. Tomoyasu, K. Yano, M. Goto, A. Murakami, E. Tsuda, T. Morinaga, K. Higashio, N. Udagawa, N. Takahashi, T. Suda, Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and...
Overexpression of sirtuin 6 suppresses inflammatory responses and bone destruction in mice with collagen-induced arthritis, Arthritis Rheum. 65 (2013) 1776–1785.

Comparison of (-)-epigallocatechin-3-gallate elicited protective effects of liver and small intestine gene expression profiles between C57BL/6J mice and C57BL/6J/Nrf2 (-/-) mice, Pharm. Res. 22 (2005) 1805–1820.

Curcumin activates the haem oxygenase-1 gene via regulation of Nrf2 and the antioxidant-responsive element, Biochem. J. 371 (2003) 887–895.

KIAA0132 and Nrf2 mediate indomethacin-induced expression of gamma-glutamylcysteine synthetase, Free Radic. Biol. Med. 32 (2002) 650–662.

(-)-Epigallocatechin-3-gallate suppresses osteoclast differentiation and ameliorates experimental arthritis in mice, Arthritis Rheum 58 (2008) 2012–2018.

The ovariectomized, mature rat model of osteoclast differentiation, Arthritis Rheum. 65 (2013) 1776–1785.