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A. **Nota de constatare şi confirmare a indiciilor de plagiat** prin fişa suspiciunii inclusă în decizie.

Fişa suspiciunii de plagiat / Sheet of plagiarism's suspicion		
Opera suspicionată (OS)		**Opera autentică (OA)**
Suspicious work		**Authentic work**
OS	DINU, Gabriel Ovidiu. New insights in osteoporosis mechanisms as common complication in Alzheimer's disease (AD) patients. *Galenus*. Ianuarie - Februarie 2015. pp.42-45. Sursa suspiciunii: Pandora2 / 13 decembrie 2017 / www.antiplagiarism2014blog2. wordpress.com.	
OA	CORNELIUS, Carolin; KOVERECH, Guido ; CRUPI, Rosanna; Di PAOLA; KOVERECH, Angela; LODATO, Francesca; SCUTO, Maria; SALINARO, T. Angela; CUZZOCREA, Salvatore; CALABRESE, J. Edward; and CALABRESE, Vittorio. Osteoporosis and Alzheimer pathology: role of cellular stress response and hormetic redox signaling in aging and bone remodeling. *Frontiers in Pharmacology | Experimental Pharmacology and Drug Discovery*. doi: 10.3389/fphar.2014.00120. June 2014. **5**(120). pp.1-13. Available at: www.frontiersin.org. Last visit: December 15, 2017.	
Incidenţa minimă a suspiciunii / Minimum incidence of suspicion		
P01[1]	p.42:02d - p.42: 17d	p.07:44s - p.07:53
P02	p.42:22d - p.44: 22m	p.07:54s - p.08:48d
Fişa întocmită pentru includerea suspiciunii în Indexul Operelor Plagiate în România de la Sheet drawn up for including the suspicion in the Index of Plagiarized Works in Romania at www.plagiate.ro		

Notă: Prin „p.72:00" se înţelege paragraful care se termină la finele pag.72. Notaţia „p.00:00" semnifică până la ultima pagină a capitolului curent, în întregime de la punctul iniţial al preluării.

Note: By „p.72:00" one understands the text ending with the end of the page 72. By „p.00:00" one understands the taking over from the initial point till the last page of the current chapter, entirely.

B. **Fişa de argumentare a calificării** de plagiat alăturată, fişă care la rândul său este parte a deciziei.

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[1] Pn este numărul piesei de creaţie care constituie obiectul preluării neconforme / Pn is the number of the creative piece, object of the non-conforming takeover.

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Fişa de argumentare a calificării

Nr. crt.	Descrierea situaţiei care este încadrată drept plagiat	Se confirmă
1.	Preluarea identică a unor pasaje (piese de creaţie de tip text) dintr-o operă autentică publicată, fără precizarea întinderii şi menţionarea provenienţei şi însuşirea acestora într-o lucrare ulterioară celei autentice.	✓
2.	Preluarea a unor pasaje (piese de creaţie de tip text) dintr-o operă autentică publicată, care sunt rezumate ale unor opere anterioare operei autentice, fără precizarea întinderii şi menţionarea provenienţei şi însuşirea acestora într-o lucrare ulterioară celei autentice.	
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11.	Preluarea identică a textului (piese de creaţie de tip text) unei lucrări publicate anterior sau simultan, cu acelaşi titlu sau cu titlu similar, de un acelaşi autor / un acelaşi grup de autori în publicaţii sau edituri diferite.	
12.	Preluarea identică de pasaje (piese de creaţie de tip text) ale unui cuvânt înainte sau ale unei prefeţe care se referă la două opere, diferite, publicate în două momente diferite de timp.	

Notă:

a) Prin „provenienţă" se înţelege informaţia din care se pot identifica cel puţin numele autorului / autorilor, titlul operei, anul apariţiei.

b) Plagiatul este definit prin textul legii[2].

> *„ …plagiatul – expunerea într-o operă scrisă sau o comunicare orală, inclusiv în format electronic, a unor texte, idei, demonstraţii, date, ipoteze, teorii, rezultate ori metode ştiinţifice extrase din opere scrise, inclusiv în format electronic, ale altor autori, fără a menţiona acest lucru şi fără a face trimitere la operele originale…".*

Tehnic, plagiatul are la bază conceptul de **piesă de creaţie** care[3]:

> *„…este un element de comunicare prezentat în formă scrisă, ca text, imagine sau combinat, care posedă un subiect, o organizare sau o construcţie logică şi de argumentare care presupune nişte premise, un raţionament şi o concluzie. Piesa de creaţie presupune în mod necesar o formă de exprimare specifică unei persoane. Piesa de creaţie se poate asocia cu întreaga operă autentică sau cu o parte a acesteia…"*

cu care se poate face identificarea operei plagiate sau suspicionate de plagiat[4]:

> *„…O operă de creaţie se găseşte în poziţia de operă plagiată sau operă suspicionată de plagiat în raport cu o altă operă considerată autentică dacă:*
> *i) Cele două opere tratează acelaşi subiect sau subiecte înrudite.*
> *ii) Opera autentică a fost făcută publică anterior operei suspicionate.*
> *iii) Cele două opere conţin piese de creaţie identificabile comune care posedă, fiecare în parte, un subiect şi o formă de prezentare bine definită.*
> *iv) Pentru piesele de creaţie comune, adică prezente în opera autentică şi în opera suspicionată, nu există o menţionare explicită a provenienţei. Menţionarea provenienţei se face printr-o citare care permite identificarea piesei de creaţie preluate din opera autentică.*
> *v) Simpla menţionare a titlului unei opere autentice într-un capitol de bibliografie sau similar acestuia fără delimitarea întinderii preluării nu este de natură să evite punerea în discuţie a suspiciunii de plagiat.*
> *vi) Piesele de creaţie preluate din opera autentică se utilizează la construcţii realizate prin juxtapunere fără ca acestea să fie tratate de autorul operei suspicionate prin poziţia sa explicită.*
> *vii) In opera suspicionată se identifică un fir sau mai multe fire logice de argumentare şi tratare care leagă aceleaşi premise cu aceleaşi concluzii ca în opera autentică…"*

[2] Legea nr. 206/2004 privind buna conduită în cercetarea ştiinţifică, dezvoltarea tehnologică şi inovare, publicată în Monitorul Oficial al României, Partea I, nr. 505 din 4 iunie 2004
[3] ISOC, D. *Ghid de acţiune împotriva plagiatului: bună-conduită, prevenire, combatere*. Cluj-Napoca: Ecou Transilvan, 2012.
[4] ISOC, D. *Prevenitor de plagiat*. Cluj-Napoca: Ecou Transilvan, 2014.

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Osteoporosis and Alzheimer pathology: role of cellular stress response and hormetic redox signaling in aging and bone remodeling

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Alzheimer's disease (AD) and osteoporosis are multifactorial progressive degenerative disorders. Increasing evidence shows that osteoporosis and hip fracture are common complication observed in AD patients, although the mechanisms underlying this association remain poorly understood. Reactive oxygen species (ROS) are emerging as intracellular redox signaling molecules involved in the regulation of bone metabolism, including receptor activator of nuclear factor-κB ligand-dependent osteoclast differentiation, but they also have cytotoxic effects that include lipoperoxidation and oxidative damage to proteins and DNA. ROS generation, which is implicated in the regulation of cellular stress response mechanisms, is an integrated, highly regulated, process under control of redox sensitive genes coding for redox proteins called vitagenes. Vitagenes, encoding for proteins such as heat shock proteins (Hsps) Hsp32, Hsp70, the thioredoxin, and the sirtuin protein, represent a systems controlling a complex network of intracellular signaling pathways relevant to life span and involved in the preservation of cellular homeostasis under stress conditions. Consistently, nutritional anti-oxidants have demonstrated their neuroprotective potential through a hormetic-dependent activation of vitagenes. The biological relevance of dose–response affects those strategies pointing to the optimal dosing to patients in the treatment of numerous diseases. Thus, the heat shock response has become an important hormetic target for novel cytoprotective strategies focusing on the pharmacological development of compounds capable of modulating stress response mechanisms. Here we discuss possible signaling mechanisms involved in the activation of vitagenes which, relevant to bone remodeling and through enhancement of cellular stress resistance provide a rationale to limit the deleterious consequences associated to homeostasis disruption with consequent impact on the aging process.

Keywords: oxidative stress, redox status, Alzheimer's disease, cellular stress response, hormesis, vitagenes

INTRODUCTION

Mitochondrial medicine is emerging as powerful candidate for expanding anatomical and Mendelian biological concepts aimed to solve complexity in age-related diseases, aging and cancer (Di Domenico et al., 2010; Wallace, 2010, 2012, 2013). Interaction between structure and energy is a fundamental life prerequisite. This dualism in eukaryotic cell, was generated approximately two billion years ago after the symbiosis of a glycolytic progenitor, which evolved the nucleus–cytosolic compartment, and an oxidative progenitor evolving toward an ancient mitochondrion. Initially each proto-organism contained all the genes for an independent life. However, 1.2 billion years later, after subsequent genomic reorganizations and alternative rearrangements, was achieved a cellular arrangement in which the mitochondrial compartment became specialized in the generation of energy, while the nuclear and cytosolic compartment was functionally polarized toward structure. This final arrangement was the starting point for multicellularity fostering evolution in higher plants and animal kingdom, including humans. Notably, this original architecture comeback powerfully in our cells in all conditions where tumor initiation and promotion occur and the glycolytic tone of metabolic potential put in motion a process leading to the marginalization of energy transduction mechanisms, kicking off mitochondrial energy production in favor of a sustained high proliferative potential which underlie tumor progression (Calabrese et al., 2007c; Wallace, 2008; Perluigi et al., 2010; Bellia et al., 2011). In view of this, comprehension of aging mechanisms of aging and determinants of life span will contribute to decrease age-related morbidity and promote healthy aging. Over the last centuries, as a consequence of exogenous environmental factors

it is now believed that the modest hormetic stimulation is a measure of biological plasticity it suggests that the hormetic dose responses describes the degree to which pharmaceutical agents can enhance biological performance (Calabrese and Mattson, 2011; Calabrese, 2013a). It is important to note that during the entire twentieth century the biomedical and regulatory communities never validated the capacity of the threshold dose response to make accurate predictions below the threshold, that is, the area of the dose response where most people spend the majority of their time. This lead to a series of direct, head-to-head comparisons between the threshold, linear and hormetic dose response models using multiple large and independent dose response data sets with multiple models, endpoints and agents (Calabrese and Baldwin, 2001, 2003; Calabrese et al., 2006a, 2008a, 2010a). In each of these assessments, the hormetic dose response significantly out-performed the threshold and linear dose response models.

Major comprehensive evaluations of the biomedical literature have revealed that hormesis dose responses are commonly reported in essentially all areas of research including the immune system, tumor cell biology, neuroscience, including memory, stress, anxiety, seizure, pain, numerous degenerative diseases, wound healing and for a very broad range of receptor systems and peptides (Calabrese, 2013b). The applications of the hormesis dose response are therefore extensive, affecting drug discovery, drug development, and the design of the clinical trial. Furthermore, even areas such as preconditioning are based on the hormetic concept (Calabrese et al., 2010b,c, 2012a, 2013; Calabrese and Calabrese, 2013a,b; Krenz et al., 2013). This may be seen in numerous studies which have assessed a wide range of graded preconditioning doses with results being reflective of the biphasic-hormetic dose response. The historical assessment of the dose response has revealed that the dose response model that was once rejected by the biomedical community about a century ago is now the dose response model that has repeatedly outcompeted the traditional doses responses models in direct comparisons. It is the dose response model that has provided quantitative insight into the magnitude of biological plasticity and the theoretical basis for it. The hormetic dose response thus underlines the basis of pharmaceutical strategies aimed at enhancing biological performances in a broad range of scientific areas.

BONE REMODELING, REDOX STATE, AND Aβ METABOLISM

P01 Osteoporosis is a devastating disease having enormous health and economic impacts, particularly considering the global shift toward an aging population, characterized by a systemic and progressive skeletal pathology characterized by compromised bone mineral density and strength with the increased occurrence of fractures. Despite rapid progress in our understanding over recent years, patient morbidity and mortality resulting from this disease are still too high (Ono et al., 2014), and there is an urgent need for a proper assessment of the underlying mechanisms and the development of new treatment strategies to address this pathophysiological issue.

P02 Patients with AD show significantly increased risk of osteoporotic hip fractures. However, whether abnormal Aβ peptide (Aβ) deposition also occurs in osteoporosis and the relationship between Aβ and human osteoporosis remains an open, not elucidated, question (Li et al., 2014).

Amyloid-β peptide, one of the pathological hallmarks of AD, is a small (40–42 amino acids) proteolytic fragment of a glycosylated integral membrane cell surface receptor protein called APP and is encoded by a gene on human chromosome 21 (Liu and Chan, 2014; Richard and Brayne, 2014). Aβ has attracted much attention for its association with various pathologies (Stefanova et al., 2014). Besides AD, Aβ plays a crucial role in other important neurodegenerative diseases, such as Huntington's, Parkinson's, and prion disorders, as well as amyotrophic lateral sclerosis as well as type 2 diabetes and the most common age-related muscle disease of inclusion body myositis (Tóth et al., 2014). Thus, APP/Aβ seems to be associated with multiple degenerative disorders. Epidemiological studies showed that patients with AD had an increased risk of developing osteoporotic hip fractures even after considering the increased frequency of fallings in AD patients (Xia et al., 2013), suggesting one or more common denominators between both disorders. Nevertheless, an association between Aβ and human osteoporosis has not yet been clearly established, and also it has been inferred that Aβ may be of physiological importance for survival of cells (Li et al., 2014). Excessive Aβ aggregates and fibrillates to form amyloid plaques in the brain, thus leading to the exacerbation of AD pathology. Previous studies have identified a role for Aβ in the activation of osteoclasts through gene knockout experiments and use of the transgenic AD mouse model, Tg2576 (Li et al., 2014). However, whether a large amount of Aβ deposits also occur in osteoporotic bone tissues and the role human Aβ may play on OC activation remain unclear. In addition to having an activation effect on osteoclasts, Aβ may accumulate abnormally in osteoporotic bone and play an important pathogenic role. A close relationship between Aβ and osteoporosis is shown across species from rodent to human, as demonstrated in different clinical conditions in patient samples as well as in various animal model and cell cultures. AD and osteoporotic hip fractures often coexist during aging. Platelets have been shown to be the primary source (90%) of Aβ in human blood with plasma Aβ levels fluctuating over time among individuals (Roher et al., 2009). Besides human plasma and cerebrospinal fluid, soluble Aβ is also a component of human urine in Alzheimer's. Despite this level of knowledge surrounding APP and Aβ expression in many tissues, reports on the expression and distribution of Aβ in bone tissues and osteocytes remain an emerging evidence. Aβ deposition has been found on the endosteal and periosteal surfaces of adult rat ulnae (Li et al., 2014). Notably, occurrence of Aβ and APP abnormal accumulation in different tissues supports the hypothesis that Aβ diseases may be a systemic disease, suggesting that these malfolded proteins may either be produced locally in diverse organs or may originate from a common circulating precursor. Consistent with this notion, abnormal Aβ and APP burden has been detected in osteoporotic bone tissues from both human and rat OVX models, where Aβ42 was identified mainly in the membrane and cytoplasm of osteocytes and extracellular matrix, while APP largely found in the membrane of osteocytes. Despite increasing research efforts, still the mechanism underlying the accumulation of Aβ and APP in osteocytes in osteoporotic bones remains elusive. One possible source for

Aβ deposition in bone may be blood, where Aβ increases during senescence (Li et al., 2014). In addition to this, secretion of Aβ by mature osteoblasts has been documented, in agreement with the finding of Aβ42 and APP formation in osteoblasts from both human and OVX rats osteoporotic bone. In these conditions, APP has been found to be able of suppressing osteoblast differentiation, associated with osteoporotic alterations (Xia et al., 2013). Given that osteoblast is the precursor of the osteocyte, it is conceivable that the deposition of Aβ and APP in osteocytes can be consequence of secretion by osteoblasts during both osteogenic differentiation and aging processes. In turn, accumulating Aβ may promote apoptotic process in osteocytes, likewise in neurons thus determining bone loss and osteoporosis (Cui et al., 2011). An interesting and controversial question concerns why the abundant presence of proteins of Aβ abnormal metabolism in the bone of osteoporotic patients did not cause them to develop brain degeneration, such as AD. A number of explanations may exist to provide a possible rationale. First, bone is a very special organ, with limited blood supply, with most of the osteocytes embedded in the matrix without direct contact with blood. In these conditions, release of Aβ into the blood stream is not an easy process. Second, the blood–brain-barrier (BBB) permeability can be of extreme importance for the prevention of Aβ invasion into the brain tissues, as uptake of peripheral Aβ by the brain is not a normal occurrence without BBB compromission (Jefferies et al., 2013). Lastly, both osteoporosis and AD are multifactorial diseases with complex etiology and pathogenesis (Rachner et al., 2011). Aβ deposits are present in several tissues, which indicates that the protein may originate as product of local metabolism in various organs or, similarly to other amyloidoses, can derive from a circulating precursor common to all these pathophysiological conditions. However, further studies are necessary to explore these dynamics and understand the underlying mechanisms. Abnormal Aβ deposition in osteoporotic bone tissues and its potent enhancement effect on osteoclast differentiation and activation, is already clearly demonstrated suggesting an important role for Aβ in the pathogenesis of osteoporosis (Li et al., 2014). This is of great clinical significance for providing novel insights into the tight link between Aβ and human osteoporosis, thus revealing a potential mechanism underlying altered bone mineral density by Aβ abnormal metabolism. Clearly, however, further work is required to elucidate the exact mechanisms through which Aβ regulates osteoporosis signaling. These research efforts may eventually lead to a promising future discovery of a new etiology for osteoporosis, and prompt healthcare professionals and researchers to develop innovative anti-bone-resorptive therapeutic agents and strategies, particularly those designed by targeting Aβ, to efficiently minimize deleterious consequences associated with bone homeostasis disruption. In line with these evidence, since a biomarker is a traceable substance indicating changes in expression or metabolism of a given protein which correlates with the risk or progression of a disease, as consequence, Aβ may be a novel and promising candidate biomarker for drug targeting and characterization of osteoporotic therapeutic approaches in the future (Osorio et al., 2014).

Bone tissue undergoes, throughout life, a continuous renewal through a process called bone remodeling, which is controlled by the activity of osteoclasts mediating bone resorption and parallel activity of osteoblasts which mediate bone formation (Vacek et al., 2013). Any disturbance in the balanced formation and resorption process, which can be linked to hormone disequilibrium or aging decreases bone mass and result in bone pathologies, such as osteoporosis leading to increased vulnerability to fractures. Within this context, the receptor activator of NF-κB ligand (RANKL) appears to be an important factor underlying osteoporosis pathogenesis for its critical role played in osteoclast differentiation and activation (Park et al., 2014). For this reason, inhibition of RANKL represents an innovative therapeutic target for controlling osteoclastogenesis (Park et al., 2011). Notably, an important role in bone remodeling is played by alternative or non-canonical NF-κB pathway, which mediates activation of the p52/RelB NF-κB complex, thus regulating various biological processes. This pathway differently from IκBα degradation in the canonical mechanism, consists of processing of p100 a NF-κB2 precursor protein,. In this context a central role is played by NF-κB-inducing kinase (NIK), a component of the non-canonical NF-κB pathway and a downstream kinase, IKKα (inhibitor of NF-κB kinase) which operate with integrated functions promoting induction of phosphorylation-dependent ubiquitination of p100. Under normal conditions, NIK is processed by a tumor necrosis factor (TNF) receptor-associated factor-3 (TRAF3)-dependent E3 ubiquitin ligase. After signals mediated by a subset of TNF receptor superfamily members, TRAF3 is degradated and NIK is stabilized leading to non-canonical activation of NF-κB (Sun, 2012; **Figure 3**). Accordingly, the inhibitory role of p100, in both basal and stimulated osteoclastogenesis in bone formation as well as resorption has been clearly demonstrated (Soysa et al., 2010). In the alternative NF-κB pathway p52 derived from p100 through NIK, binding of p52 and RelB induces effects on osteoclast biology (Soysa et al., 2010). However, to date, the precise physiologic importance of alternative NF-κB in bone biology, is not completely elucidated. Furthermore, the currently known intracellular signaling pathways activated after receptor binding of RANKL include the nuclear factor of activated T cells (Piva et al., 2009), mitogen-activated protein kinases (MAPKs), TRAFs, c-Jun N-terminal kinases (JNKs), and ROS (Kaunitz and Yamaguchi, 2008; Kanzaki et al., 2013). In addition, NF-κB is a transcription factor, which pleiotropically regulate osteoclast formation, function, and survival (Piva et al., 2009). Deletion of both NF-κB p50 and p52 subunits is associated to osteopetrosis as consequence of osteoclast absence and, in addition, NF-κB is central for the differentiation of RANK-expressing osteoclasts into osteoclasts $TRAP^{+}$ induced by osteoclastogenic cytokines. This explain the inhibitory effect on osteoclast formation induced by prevention of NF-κB activation (Piva et al., 2009; Augustine and Horwitz, 2013).

Reactive oxygen species act as intracellular signaling molecules involved in the regulation of RANKL-dependent osteoclast differentiation, but they also have cytotoxic effects that include peroxidation of lipids and oxidative damage to proteins and DNA. Taking into account the relationship between Nrf2 and osteoclastogenesis, stimulation of osteoclast precursors (mouse primary peritoneal macrophages and RAW 264.7 cells) with RANKL results in the up-regulation of Keap1, a negative regulator of Nrf2, with decreased

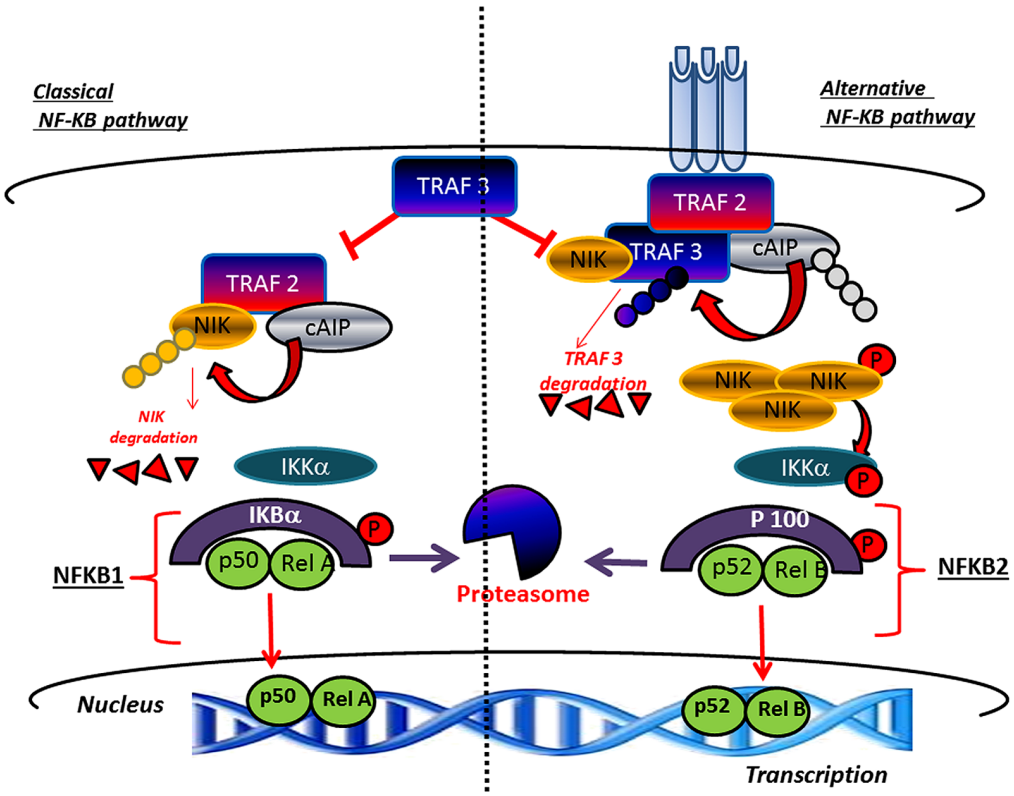

FIGURE 3 | Canonical and non-canonical pathway leading to the activation of NF-κB. TRAF3 inhibits activation of the classical NFκB pathway A high level of TRAF3 interferes with the recruitment of TRAF2 to the receptor. In parallel, TRAF3 induces NIK degradation and consequently inhibits the activation of the alternative arm of NFκB. The activation of canonical pathway results in the phosphorylation of IκBαα by the IKK complex, leading to its ubiquitylation and subsequent degradation by the proteasome. The RelA/p50 complex is free to translocate to the nucleus to activate the transcription of target genes. The non-canonical pathway results in the NIK stabilization. In response to receptor crosslinking, TRAFs and cIAP1/2 are recruited to the receptor, where cIAP1/2 ubiquitinates TRAF2 and TRAF3 and stimulates their degradation. Accumulated NIK activates IKKα, which in turn phosphorylates p100, leading to p100 processing to p52, which can lead to the activation of p52-RelB that target distinct κB element and induce the transcription of target genes.

Nrf2/Keap1 ratio, and down-regulation of cytoprotective enzymes, such as heme oxygenase-1 and γ-glutamylcysteine synthetase (Kanzaki et al., 2013). On the other hand, Nrf2 overexpression results in up-regulation of the expression of cytoprotective enzymes, associated with decrease in ROS levels, tartrate-resistant acid phosphatase-positive multinucleated cell number, as well as osteoclast differentiation, and attenuation of bone destruction, as found both *in vitro* and *in vivo* models (Kanzaki et al., 2013). Consistent with this line of evidence, overexpression of Keap1 or RNAi-induced knock-down of Nrf2 resulted in effects opposite to those obtained by stimulation of Nrf2-dependent DNA binding activity (Kanzaki et al., 2013). The precise mechanisms by which stimulation with RANKL reduces Nrf2 is not currently known. It is known Keap1 has highly reactive thiol groups in its structure and that oxidation of this domain leads to significant changes in the conformation of Keap1, resulting in dissociation from Nrf2 and stimulation of nuclear Nrf2-dependent DNA binding activity (Kanzaki et al., 2013). In addition, Nrf2 (see previous section) autoregulates its own expression (Calabrese et al., 2010d; Zhang et al., 2011, 2014). Taken together, this evidence implies that an increase in ROS levels induced by stimulation with RANKL may up-regulate Nrf2. It has also been reported that Nrf2 regulates Keap1 by controlling its transcription (Calabrese et al., 2010d; Zhang et al., 2014). Change of stability of Nrf2 mRNA or decrease of translation by miRNA can modulate RANKL-dependent Nrf2 down-regulation. Also, Bach1, an inhibitor of Nrf2 binding to the ARE, could participate to this mechanism, as indicated by attenuated osteoclastogenesis found in Bach1 knock-out mice (Kanzaki et al., 2013). However, although extensive investigations will be required to clarify the exact regulatory mechanisms linking Nrf2 to stimulation with RANKL, it is clearly proven that Keap1/Nrf2 axis regulates RANKL-dependent osteoclastogenesis through redox-modulation of intracellular ROS signaling and expression of cytoprotective enzymes. This raises the exciting possibility that the Keap1–Nrf2 axis may be a therapeutic target for the treatment of bone destructive disease.

CONCLUSION

Comprehensive evaluation of the biomedical literature has pinpointed the role of hormetic dose responses as reported in essentially all areas of research, including the immune system,