Effect of GNAQ alteration on RANKL-induced osteoclastogenesis in human non-small-cell lung cancer

Aims
Receptor activator of nuclear factor-κB ligand (RANKL) is a key molecule that is expressed in bone stromal cells and is associated with metastasis and poor prognosis in many cancers. However, cancer cells that directly express RANKL have yet to be unveiled. The current study sought to evaluate how a single subunit of G protein, guanine nucleotide-binding protein G(q) subunit alpha (GNAQ), transforms cancer cells into RANKL-expressing cancer cells.

Methods
We investigated the specific role of GNAQ using GNAQ wild-type cell lines (non-small-cell lung cancer cell lines; A549 cell lines), GNAQ knockdown cell lines, and patient-derived cancer cells. We evaluated GNAQ, RANKL, macropage colony-stimulating factor (M-CSF), nuclear transcription factor-κB (NF-κB), inhibitor of NF-κB (IκB), and protein kinase B (Akt) signalling in the GNAQ wild-type and the GNAQ-knockdown cells. Osteoclastogenesis was also evaluated in both cell lines.

Results
In the GNAQ-knockdown cells, RANKL expression was significantly upregulated (p < 0.001). The expression levels of M-CSF were also significantly increased in the GNAQ-knockdown cells compared with control cells (p < 0.001). GNAQ knockdown cells were highly sensitive to tumour necrosis factor alpha (TNF-α) and showed significant activation of the NF-κB pathway. The expression levels of RANKL were markedly increased in GNAQ mutant compared with GNAQ wild-type in patient-derived tumour tissues.

Conclusion
The present study reveals that the alterations of GNAQ activate NF-κB pathway in cancers, which increase RANKL and M-CSF expression and induce osteoclastogenesis in cancers.

Cite this article: Bone Joint Res. 2020;9(1):29–35.

Keywords: GNAQ, RANKL, Osteoclastogenesis, Bone, Lung cancer

Article focus
- The detailed mechanisms of guanine nucleotide-binding protein G(q) subunit alpha (GNAQ) in the signal transduction pathway responsible for receptor activator of nuclear factor-κB ligand (RANKL) are not yet understood.
- This study investigated the significant roles of GNAQ in osteoclastogenesis.

Key messages
- The study showed that the expression level of RANKL and the number of osteoclasts were significantly increased in GNAQ knockdown cells (p < 0.001).

Strengths and limitations
- To our knowledge, this is the first study to assess whether suppression of GNAQ can transform lung cancer cells into RANKL-expressing cells.
- The absence of an in vivo model is a principal limitation of this study.

Introduction
Refractory bone metastasis, for which all conventional treatments such as chemotherapy and radiation therapy have failed, often results in pathological fractures. Efforts have been made to prevent pathological fractures of metastatic bone. Bisphosphonates are...
commonly used to reduce the risk of pathological fractures. A recent study showed that denosumab, which targets receptor activator of nuclear factor-κB ligand (RANKL) and is also known as tumour necrosis factor (TNF) ligand superfamily member 11 (TNFSF11) or TNF-related activation-induced cytokine (TRANCE), prevents or delays skeletal-related events (SREs) and can improve a prognosis.1–3

Receptor activator of nuclear factor-κB ligand, a cell membrane-bound TNF superfamily member, binds to receptor activator of nuclear factor-κB (RANK) expressed on osteoclast precursors, which then leads to the fusion, differentiation, and maturation of osteoclasts.4 The RANK/RANKL/osteoprotegerin (OPG) system is a master regulator of the bone resorption process by activating the osteoclasts. OPG functions as a decoy receptor for RANKL, which inhibits RANKL-induced osteoclast differentiation.5,6 RANKL is expressed at a high level in stromal cells. Therefore, cancer cells indirectly induce RANKL expression via stromal cells, which accelerates bone metastasis. Interestingly, some cancers have been observed to express RANKL by themselves.7,8

RANKL-expressing cancers are correlated with poor prognosis. Among gastric cancer patients, RANKL expression was observed in 33% of the patients with a poor prognosis.9 Patients with renal cell carcinoma which expresses high levels of RANKL showed shorter bone metastasis-free survival and disease-free survival.10 Breast cancer patients with RANKL-positive primary tumours exhibited poorer clinical outcomes than patients with RANKL-negative primary tumours.2 In addition, inhibition of RANKL has been shown to improve the overall survival in patients with metastatic lung cancer. However, the mechanisms by which cancer cells transform to RANKL-expressing cells have yet to be fully researched.

Guanine nucleotide-binding proteins (G proteins) and G protein-coupled receptors (GPCRs) transduce extracellular signals and involve multiple processes of mammary cells including hormonal signal transduction, metabolism, cell survival, and sensory functions.12,13 The heterotrimeric G proteins of α, β, and γ subunits provide the specificity and functionality of GPCRs in a cell type- and tumour-specific way. Guanine nucleotide-binding proteins are classified into four subfamilies: Gαq, Gα12/13, and Gα11. Gαq is encoded by the GNAQ gene.14,15 GNAQ mutations have been associated with several carcinomas.16,17 About 85% of melanoma patients presenting metastasis and higher rates of mortality exhibit mutations in GNAQ.10,18 In the current study, we found that the alteration of GNAQ induced RANKL expression in lung cancer cells. This study aimed to determine how this GNAQ alteration is involved in the signal transduction pathway responsible for RANKL expression.

Methods

Patient samples. Primary tumour cells were obtained from patients who underwent surgery at the Samsung Medical Center, Sungkyunkwan University, Seoul, South Korea, and signed the informed consent form according to the relevant guidelines and the regulation for the cell storage. A total of six lung cancer tissues obtained from metastatic bone lesion were included in the present study (Supplementary Table i).

Cell culture. Non-small-cell lung cancer (NSCLC) A549 cell lines were purchased from the American Type Culture Collection (Manassas, Virginia, USA). A549 cells were cultured in RPMI1640 medium (HyClone Laboratories, Logan, Utah, USA) supplemented with 10% fetal bovine serum, and 1% antibiotic-antimycotic (Gibco, Waltham, Massachusetts, USA). Cell lines were incubated in a 5% CO2-humidified atmosphere at 37°C. RAW 264.7 cells were grown in Dulbecco’s Modified Eagle Medium (DMEM) (HyClone Laboratories) with 10% fetal bovine serum and 1% antibiotic-antimycotic.

Evaluation of the GNAQ status. The GNAQ status of the samples was evaluated according to the same process as we described in the previous study.19 Briefly, the genomic status of GNAQ was screened using CancerSCAN, a Next-Generation-Sequencing-based analysis which used Illumina HiSeq 2000 (Illumina, San Diego, California, USA). Sanger sequencing was used as a confirmative method to find a specific genetic alteration.

Reverse transcription polymerase chain reaction and quantitative real-time polymerase chain reaction analysis. Total RNA isolated from the cultured cells was reverse-transcribed using a SuperScriptIII cDNA Synthesis kit (Life Technologies, Carlsbad, California, USA). Polymerase chain reaction (PCR) was performed using AccuPower® Hotstart PCR PreMix (Bioneer, Daejeon, Korea), and quantitative real-time PCR (qRT-PCR) was carried out in the Applied Biosystems 7900HT (Applied Biosystems, Foster City, California, USA). The target genes were amplified using the following primers: GNAQ forward, 5′-GCACAAATAAGGCTCAGAC-3′ and reverse, 5′-TTGGGACCCAGGTTATGAT-3′; RANKL forward, 5′-TATGCCAATACATTCGTCG-3′ and reverse, 5′-CTCGGGATTATGCTGATT-3′; macrophage colony-stimulating factor (M-CSF) forward, 5′-AGCGAGGATCACCCGAGGA-3′ and reverse, 5′-TATTTGGCAGGATTCCTC-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward, 5′-CATGACATCAAGAGTGG-3′ and reverse, 5′-TCCCTGCGCAGGTGC-3′ and reverse, 5′-GCAGTGATCTCC TTCTGCAT-3′.

---

**J.Y. Choi, Y. S. Lee, D. M. Shim, S. W. Seo**

**BONE & JOINT RESEARCH**
**Western blotting.** The cells were lysed in radioimmunoprecipitation assay (RIPA) buffer supplemented with protease inhibitors (Thermo Fisher Scientific, Piscataway, New Jersey, USA). Protein supernatants were obtained by centrifugation at 14,000 × g for 15 minutes at 4°C. Protein quantification was performed using a bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific) according to the manufacturer’s protocol. The protein samples were separated using sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Hercules, California, USA) for western blotting. The membrane was blocked with 5% skim milk for one hour and then immunoblotted with a primary antibody, GNAQ (Abcam, Cambridge, UK), RANKL, β-actin (Santa Cruz Biotechnology, Dallas, Texas, USA), or β-tubulin (Cell Signaling Technology, Danvers, Massachusetts, USA), at a dilution of 1:1,000 overnight at 4°C.

**Osteoclastogenesis assay.** RAW 264.7 cells were seeded in 24-well plates at a density of 2 × 10^3 cells per well. Negative control lentiviral vector (A549_shNC) and GNAQ short hairpin RNA lentiviral vector (A549_shGNAQ) cells were subsequently seeded in minimum essential medium-alpha modification (α-MEM) (HyClone Laboratories) overlaying the RAW 264.7 cells at a density of 2 × 10^4 cells per well. Cells were treated with RANKL (50 ng/ml) and M-CSF (20 ng/ml) (R & D Systems, Minneapolis, Minnesota, USA) to stimulate osteoclast differentiation. After five days, the cells were fixed and stained for tartrate-resistant acid phosphatase (TRAP) activity using a TRAP assay Kit (Takara Bio, Mountain View, California, USA), as per the manufacturer’s instructions. For quantification, osteoclasts were defined as multinucleated (more than three nuclei) TRAP-positive cells.9 Cells were visualized at 200 × magnification using a light microscope. Electronic images of five pre-determined areas per well were obtained, and TRAP-positive cells were counted in each image.

**Statistical analysis.** The results were presented as means and standard deviations (SDs). The p-values were evaluated using Mann-Whitney test and paired t-test in GraphPad Prism5 (GraphPad Software, La Jolla, California, USA). Statistical significance was set at p < 0.05.

**Results**

**GNAQ knockdown induces osteoclastogenesis.** To investigate the role of GNAQ in osteoclastogenesis, we performed knockdown experiments using short hairpin RNA (shRNA) specific to GNAQ. The human lung adenocarcinoma A549 cells were transfected with shGNAQ. We found that both the messenger RNA (mRNA) and protein levels of GNAQ were decreased in the shGNAQ compared with the shNC group (Figure 1). We have successfully established a stable A549_shGNAQ cell line for further studies, which is confirmed using qRT-PCR and western blotting.

**RANKL activates the RANK receptor on the surface of osteoclast precursor cells and promotes their differentiation into multinucleated giant cells.**20 Our qRT-PCR revealed that mRNA expression of RANKL was significantly (p < 0.001) increased in GNAQ knockdown cells compared with that expressed in control cells (Figure 2a). These results were validated using semi-quantitative PCR (Figure 2b). Western blotting analysis showed that RANKL was markedly increased in GNAQ knockdown cells (Figure 2c). We investigated whether GNAQ knockdown cells can stimulate osteoclastogenesis in an osteoclast precursor cell line, RAW 264.7. A high number of TRAP-positive cells and the higher TRAP activity were observed in a co-culture of RAW 264.7 cells and GNAQ knockdown cancer cells. However, the wild-type GNAQ cells failed to induce differentiation of RAW 264.7 cells into TRAP-positive osteoclasts (Figures 2d and 2e). Taken together, these results indicated that the suppression of GNAQ influences osteoclast formation by increasing the expression of RANKL. Moreover, the expression of M-CSF was significantly (p < 0.001) elevated up to 5.3-fold in the GNAQ knockdown cells compared with that in the control cells (Figure 3). These results indicated that the suppression of GNAQ regulates osteoclastogenesis by inducing the expression of RANKL and M-CSF in cancer cells.

**Effect of GNAQ knockdown on protein kinase B (Akt)/nuclear transcription factor-κB (NF-κB) signalling pathway.** As noted in many studies,21-23 TNF-α, a major...
Induction of receptor activator of nuclear factor-κB ligand (RANKL) by knockdown in guanine nucleotide-binding protein G(q) subunit alpha expression (GNAQ). The expression level of RANKL mRNA was determined by a) real-time polymerase chain reaction and b) semi-quantitative polymerase chain reaction. c) Protein levels of RANKL were determined by western blotting. d) Representative images of tartrate-resistant acid phosphatase (TRAP) staining after induction of osteoclastogenesis by co-culture of RAW 264.7 cells with A549, short hairpin RNA negative control lentiviral vector (A549_shNC), or GNAQ short hairpin RNA lentiviral vector (A549_shGNAQ) cells. Cells were 1:1 co-cultured and treated with RANKL (50 ng/ml) and macrophage colony-stimulating factor (M-CSF, 20 ng/ml). The number of TRAP-positive cells was higher in the GNAQ knockdown group than in the control group. Images were captured using a light microscope (magnification ×40 or ×200). e) Evaluation of the number of TRAP-positive cells (n = 3). Error bars represent mean (SD). *Statistically significant (p < 0.001). †Statistically significant (p < 0.05). GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
**Effect of GNAQ alteration on RANKL-induced osteoclastogenesis in human non-small-cell lung cancer**

Pro-inflammatory cytokine, plays an important role in RANKL expression via the nuclear transcription factor-κB (NF-κB) signalling pathway. Therefore, to determine the effect of GNAQ on the NF-κB signalling pathway, NF-κB signalling was evaluated using western blot. We found that the expression levels of inhibitor of NF-κB (IkB) were reduced in GNAQ knockdown cells. In contrast, IkB was activated in the control cells two hours after the administration of TNF-α. We observed that the suppression of GNAQ activated NFκB signalling pathway by inducing phosphorylation of NFκB-p65 protein and protein kinase B (Akt) (Figure 4). Furthermore, the phosphorylation levels of Akt and NF-κB were increased upon the administration of TNF-α in GNAQ knockdown cells. Our results indicate that suppression of GNAQ facilitated Akt/NF-κB signalling pathway and elevated the sensitivity to TNF-α.

**Discussion**

In this study, we found the silencing of GNAQ upregulated the NF-κB signalling pathway in lung cancer cells and increased the expression of RANKL and M-CSF. In vitro experiments showed that cancer cells with GNAQ knockdown effectively transformed macrophage (RAW 264.7) cells into TRAP-positive osteoclasts.

RANKL was initially identified on the surface of stromal cells, a major cell that mediates osteoclastogenesis. RANKL expression has been found in many tissues. It has recently been reported that human articular cartilage can express and produce RANKL. High expression of RANKL is commonly detected in the lymph nodes, thymus, and lungs. A weak level of RANKL expression can be found in bone marrow, the stomach, spleen, peripheral blood, placenta, leukocytes, heart, thyroid, or skeletal muscle. In addition, RANKL expression was found in several malignant tumour cells and is localized on the surface of activated T-cell lymphocytes. RANKL expression is upregulated during the progression of prostatic carcinoma in bone. Breast cancer induces osteolytic metastases in bone by inducing RANKL expression. In renal cell carcinoma, RANKL promotes cancer cells to metastasize to bone.
In addition, it has been reported that lung cancer cells that metastasize to bone showed increased levels of RANKL. However, the mechanism by which RANKL expression is induced in cancer cells has not yet been fully examined. Our study first found that GNAQ mutation can transform cancer cells into RANKL-expressing cells. A previous study demonstrated that M-CSF plays a critical role in the regulation of multiple processes including regulation of human osteoclasts. We detected the increased expression of M-CSF in GNAQ knockout cells, which is also a crucial molecule for osteoclastogenesis. Therefore, GNAQ knockout is sufficient for transforming macrophages into osteoclasts. Indeed, GNAQ knockout cells alone were sufficient to induce RAW 264.7 cells to TRAP-positive osteoclasts.

NF-κB pathway activation has been shown as a vital contributing factor to GNAQ-mediated oncogenesis. However, the alteration in GNAQ expression and the correlation of NF-κB pathway to osteoclastogenesis require further investigation. Our study confirmed that Akt/IkB/NF-κB pathway was constitutively active in GNAQ knockdown cells. In addition, we showed that GNAQ knockdown cells were highly sensitive to TNF-α.

Analysis of gene expression databases revealed that lung cancer cells harvested from metastatic bone lesions frequently harboured GNAQ mutations (MS9L, T96S, or Y101X). We also found that the RANKL expression was increased in the metastatic bone lesion where the GNAQ mutations were found. This result indicated that the alteration in GNAQ expression in lung cancer contributes to osteoclastogenesis.

Willeumier et al reported that the prognosis of NSCLC patients with bone metastasis was related to the growth transfer receptor (EGFR) mutations. However, the clinical significance of GNAQ mutations in metastatic bone cancers is still unknown. Further research should be carried out on clinically relevant signature genes that influence the prognosis and that have potential to be used in a targeted therapy. Nevertheless, our results improve our understanding of the function of GNAQ and provide a new potential target which can offer a novel therapeutic approach to osteolytic pathologies in bone metastasis.

In conclusion, our study demonstrated that RANKL expression in metastatic bone cancers is regulated by GNAQ. The alterations of GNAQ activate the NF-κB pathway in cancers, which transforms cancer cells to express RANKL and M-CSF and induces osteoclastogenesis.

### Supplementary Material

Table showing GNAQ status of patient-derived cancer cells.

### References

1. Osagie-Clouard L, Sanghani A, Coathup M, et al. Parathyroid hormone 1-34 and skeletal anabolic action: The use of parathyroid hormone in bone formation. Bone Joint Res. 2017;6(1):14-21.
2. Reyes ME, Fujii T, Branstetter D, et al. Poor prognosis of patients with triple-negative breast cancer can be stratified by RANK and RANKL dual expression. Breast Cancer Res Treat. 2017;164(1):57-67.
3. Li M, Zhang C, Yang Y. Effects of mechanical forces on osteogenesis and osteoclastogenesis in human periodontal ligament fibroblasts: A systematic review of in vitro studies. Bone Joint Res. 2019;8(1):19-31.
4. Kartsogiannis V, Zhou H, Horwood NJ, et al. Localization of RANK (receptor activator of NF-κB ligand) mRNA and protein in skeletal and extraskeletal tissues. Bone. 1998;23(5):525-534.
5. Guise TA, Yin JJ, Taylor SD, et al. Evidence for a causal role of parathyroid hormone-related protein in the pathogenesis of human breast cancer-mediated osteolysis. J Clin Invest. 1996;98(7):1544-1549.
6. Boyce BF, Xing L. Biology of RANK, RANKL, and osteoprotegerin. Arthritis Res Ther. 2007;9(Suppl 1):S1.
7. Mikami S, Katsube K, Oya M, et al. Increased RANKL expression is related to tumour migration and metastasis of renal cell carcinomas. J Pathol. 2009;214(4):530-539.
8. Peng X, Guo W, Ren T, et al. Differential expression of the RANKL/RANK/OPG system is associated with bone metastasis in human non-small cell lung cancer. PLoS One. 2013;8(3):e58361.
9. Huang B, Zhang Q, Yuan Y, et al. Sema3a inhibits the differentiation of Raw264.7 cells to osteoclasts under 2 Gy radiation by reducing inflammation. PLoS One. 2018;13(7):e0200000.
10. Rose AM, Luo R, Radia UK, et al. Detection of mutations in SF3B1, EIF1AX and GNAQ in primary orbital melanoma by candidate gene analysis. BMC Cancer. 2016;16(1):1262.
11. Scagliotti GV, Hirsh V, Siena S, et al. Overall survival improvement in patients with lung cancer and bone metastases treated with denosumab versus zoledronic acid: subgroup analysis from a randomized phase 3 study. J Thorac Oncol. 2012;7(12):1823-1829.
12. Offermanns S. G-proteins as transducers in transmembrane signalling. Prog Biophys Mol Biol. 2003;83(2):101-130.
13. Ritter SL, Hall RA. Fine-tuning of GPCR activity by receptor-interacting proteins. Nat Rev Mol Cell Biol. 2008;9(12):819-830.
14. Oldham WM, Hamm HE. Heterotrimeric G protein activation by G-protein-coupled receptors. Nat Rev Mol Cell Biol. 2008;9(11):60-71.
15. Kamato D, Thach L, Bernard R, et al. Structure, function, pharmacology, and therapeutic potential of the G protein, Gsa/q. 11. Front Cardiovasc Med. 2015;2:14.
16. Lee SH, Jung SH, Kim TM, et al. Whole-exome sequencing identified mutational profiles of high-grade colon adenomas. Oncotarget. 2017;8(41):6579-6588.
17. Zilberg C, Lee MW, Yu B, et al. Analysis of clinically relevant somatic mutations in high-risk head and neck cutaneous squamous cell carcinoma. Mod Pathol. 2018;31(2):275-287.
EFFECT OF GNAQ ALTERATION ON RANKL-INDUCED OSTEOCLASTOGENESIS IN HUMAN NON-SMALL-CELL LUNG CANCER

18. Yu J, Wu X, Yan J, et al. Potential mutations in uveal melanoma identified using targeted next-generation sequencing. J Cancer. 2019;10(2):488-493.
19. Lee YS, Choi YJ, Lee J, et al. TP53 alteration determines the combinational cytotoxic effect of doxorubicin and an antioxidant NAC. Tumour Biol. 2017;38(6):10142(317001159.
20. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. 2002;420(6937):357-362.
21. Wada N, Maeda H, Yoshimine Y, Akamine A. Lipopolysaccharide stimulates expression of osteoprotegerin and receptor activator of NF-kappaB ligand in periodontal ligament fibroblasts through the induction of interleukin-1 beta and tumor necrosis factor-alpha. Bone. 2004;3(3):629-635.
22. Pacios S, Xiao W, Mattos M, et al. Osteoblast lineage cells play an essential role in periodontal bone loss through activation of nuclear factor-kappaB. Scip Rep. 2015;5:18694.
23. Ganesan R, Doss HM, Rasool M. Majoron ushba, a polyherbal compound, suppresses pro-inflammatory mediators and RANKL expression via modulating NF-kB and MAPKs signaling pathways in fibroblast-like synoviocytes from adjuvant-induced arthritic rats. Immunol Res. 2016;64(4):1071-1086.
24. Kwan Tat S, Amible N, Pelletier JP, et al. Modulation of OPG, RANK and RANKL by human chondrocytes and their implication during osteoarthritis. Rheumatology (Oxford). 2009;48(12):1482-1490.
25. Lacey DL, Timms E, Tan HL, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell. 1998;93(2):165-176.
26. Anderson DM, Maraskovsky E, Billingsley WL, et al. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Cell. 1998;93(2):165-176.
27. Rodan GA, Martin Tj. Role of osteoblasts in hormonal control of bone resorption—a hypothesis. Calcif Tissue Int. 1981;34(4):349-351.
28. Nakashima T, Hayashi M, Fukunaga T, et al. Evidence for osteocyte osteoclastogenesis of bone homeostasis through RANKL expression. Nat Med. 2011;17(10):1231-1234.
29. Wittman Y, Theoleyre S, Chipoy C, et al. RANKL/RANK/OPG: new therapeutic targets in bone tumours and associated osteolytic. Biochim Biophysica. 2004;170(4):49-57.
30. Huang L, Cheng Y, Chow LT, Zheng MH, Kutma SM. Tumour cells produce receptor activator of NF-kappaB ligand (RANKL) in skeletal metastases. J Clin Pathol. 2002;55(11):877-878.
31. Chen G, Sircar K, Aprikiion A, Pattie A, Goltzman D, Rabbani S. Expression of RANKL/RANK/OPG in primary and metastatic human prostate cancer as markers of disease stage and functional regulation. Cancer. 2006;107(2):289-298.
32. Park H-R, Min SK, Cho HD, Kim DH, Shin HS, Park YE. Expression of osteoprotegerin and RANK ligand in breast cancer bone metastasis. J Korean Med Sci. 2003;18(4):541-546.
33. Santini D, Schiavon G, Vincenzi B, et al. Receptor activator of NF-kB (RANK) expression in primary tumors associates with bone metastasis occurrence in breast cancer patients. PLoS One. 2011;6(4):e19234.
34. Mountzios G, Dimopoulos MA, Bamias A, et al. Abnormal bone remodeling process is due to an imbalance in the receptor activator of nuclear factor-kappaB ligand (RANKL)/osteoprotegerin (OPG) axis in patients with solid tumors metastatic to the skeleton. Acta Oncol. 2007;46(2):221-229.
35. Yamashita T, Takahashi N, Udagawa N. New roles of osteoblasts involved in osteoclast differentiation. World J Orthop. 2012;3(11):175-181.
36. Wu X, Li J, Zhu M, Fletcher JA, Hodi FS. Protein kinase C inhibitor AEB071 targets ocular melanoma harboring GNAQ mutations via effects on the PKC/Erk1/2 and PKC/NF-kB pathways. Mol Cancer Ther. 2012;11(9):1805-1914.
37. Willeumier JJ, van der Hoeven NM, Bollen L, et al. Epidermal growth factor receptor mutations should be considered as a prognostic factor for survival of patients with pathological fractures or painful bone metastases from non-small cell lung cancer. Bone Jt J. 2017;99-B(4):516-521.

Author information

J.Y. Choi, PhD, Senior Researcher, Department of Orthopaedic Surgery,
Y.S. Lee, BSc, Researcher, Department of Orthopaedic Surgery,
D. M. Shim, BSc, Researcher, Department of Orthopaedic Surgery,
S. W. Seo, MD, PhD, Associate Professor, Department of Orthopaedic Surgery,
Samsung Medical Center, Sungkyunkwan University, Seoul, South Korea.

Acknowledgements

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Funding statement

This study was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education and Science Technology (MEST) (2016R1A1A0191433) and partially supported by Samsung Medical Center grant, oTC1190111.

Ethical review statement

This study was approved by the Institutional Review Board of the Samsung Medical Center (approval number: 2019-01-098).

© 2020 Author(s) et al. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (CC BY-NC-ND 4.0) licence, which permits the copying and redistribution of the work only, and provided the original author and source are credited. See https://creativecommons.org/licenses/by-nc-nd/4.0/.