Dovitinib (TKI258) is a small molecule multi-kinase inhibitor currently in clinical phase III/II development for the treatment of various types of cancers. This drug has a safe and effective pharmacokinetic/pharmacodynamic profile. Although dovitinib can bind several kinases at nanomolar concentrations, there are no reports relating to osteoporosis or osteoblast differentiation. Herein, we investigated the effect of dovitinib on human recombinant bone morphogenetic protein (BMP)-2-induced osteoblast differentiation in a cell culture model. Dovitinib enhanced the BMP-2-induced alkaline phosphatase (ALP) induction, which is a representative marker of osteoblast differentiation. Dovitinib also stimulated the translocation of phosphorylated Smad1/5/8 into the nucleus and phosphorylation of mitogen-activated protein kinases, including ERK1/2 and p38. In addition, the mRNA expression of BMP-4, BMP-7, ALP, and OCN increased with dovitinib treatment. Our results suggest that dovitinib has a potent stimulating effect on BMP-2-induced osteoblast differentiation and this existing drug has potential for repositioning in the treatment of bone-related disorders.

INTRODUCTION

In vertebrates, the balance between osteoblastic bone formation and osteoclastic bone resorption controls bone homeostasis. However, decreased osteoblastic activity and increased osteoclastic activity lead to a reduction in bone mineral density and, consequently, increases in the risk of fractures and metabolic bone disease. Decreased osteoblastic activity and increased osteoclastic bone resorption controls bone homeostasis. Thus, anabolic agents that stimulate osteoblast differentiation or antiresorptive activity have been developed to attempt to treat bone diseases (Garces and Garcia, 2006; Rosen and Bilezikian, 2001). However, since existing anabolic agents for bone diseases have some limitations in the administration methods and costs, new effective drugs are needed (Garrett, 2007). In recent years, many companies began searching existing drugs to reduce research and development (R&D) spending and adjust these drugs for other indications via the drug repositioning process (Longman, 2004; Son et al., 2013; Stuart, 2004). This process provides many pharmaceutical companies or investigators an effective way to reduce development costs and risks of failure in drug developmental processes such as pharmacokinetic/pharmacodynamic studies and clinical trials.

Dovitinib (TKI258) is a small-molecule multi-kinase inhibitor in phase III/I clinical trials for the treatment of gastric cancer, pancreatic cancer, advanced breast cancer, multiple myeloma, urothelial cancer, and renal cell carcinoma (ClinicalTrials.gov) (André et al., 2013; Hashinoff et al., 2012). Moreover, dovitinib has a safe and effective pharmacokinetic/pharmacodynamic profile (André et al., 2013; Sarker et al., 2008; Wang et al., 2013). Dovitinib was first designed and synthesized as a multi-targeted kinase inhibitor (Trudel et al., 2005). In many studies, dovitinib exerted anticancer activity and antiangiogenic activity through the inhibition of fibroblast growth factor receptor (FGFR) and platelet-derived growth factor receptor (PDGF) (Lee et al., 2005). This inhibition effect led to an in-depth investigation of dovitinib as a new anticancer drug, but there currently are no reports evaluating bone homeostasis or skeletal diseases.
In this study, we investigated whether dovitinib can regulate the BMP-2-mediated signaling pathway and exert anabolic effects in osteoblast differentiation. Our results indicated that dovitinib has potent stimulating effects in the induction of ALP and mRNA transcription of BMPs and osteogenic markers including ALP and osteocalcin (OCN) through activation of ERK1/2, p38 MAPKs, and phosphorylation of Smad1/5/8. In this study, we evaluated the effect of dovitinib as a potential anabolic agent in osteoblast differentiation of bi-potential mesenchymal precursor C2C12 cells.

**MATERIALS AND METHODS**

**Materials**

Dovitinib was purchased from Selleck Chemicals (USA) and recombinant human bone morphogenetic protein (BMP)-2 purchased from R&D Systems, Inc. (USA). All cell culture materials including fetal bovine serum (FBS), DMEM, and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) were purchased from HyClone (UK). Antibodies against p-ERK1/2, ERK1/2, p-p38, and phosphorylated Smad1/5/8 were purchased from Cell Signaling Technology, Inc. (MA, USA). An antibody against actin-horse radish peroxidase (HRP) and Smad1/5/8 were purchased from Santa Cruz Biotechnology, Inc. (USA). The ALP reagent, MEK inhibitor PD98059, and p38 inhibitor SB202190 were purchased from Sigma Aldrich (USA).

**Cell culture and differentiation**

Murine bi-potential mesenchymal precursor C2C12 (mouse myoblast cell line) cells were purchased from ATCC (USA) and maintained in DMEM containing 10% FBS and 1% antibiotics in a humidified atmosphere of 5% CO2 at 37°C. For osteoblast differentiation, C2C12 cells were seeded in a 96-well plate for 24 h. Cells were treated with BMP-2 (50 ng/ml) alone or combined with dovitinib in culture media containing 5% FBS for 72 h. Total RNA was isolated from C2C12 cells using TRIzol reagent (Life Technologies, USA) and cDNA was synthesized from 1 μg of total RNA using the Enzyrnics™ Reverse Transcriptase Kit (Enzymics, Korea), according to the manufacturer’s instructions. Quantitative real-time PCR was performed using the IQ™ SYBR Green Supermix (Bio-Rad, USA) and the CFX96™ Real-Time System (Bio-Rad, USA). The primer sequences used in this study are shown in Table 1. All reactions were run in triplicate, and data were analyzed using the 2-ΔΔC_{T} method (Livak and Schmittgen, 2001). The internal standard was GAPDH and statistical significance was determined with the Student’s t-Test using GAPDH-normalized 2^{-ΔΔC_{T}} values (Livak and Schmittgen, 2001).

**Alkaline phosphatase (ALP) activity assay**

The C2C12 cells (4 × 10³ cells/well) were seeded in a 96-well plate for 24 h. Cells were treated with BMP-2 (50 ng/ml) alone or combined with dovitinib in culture media containing 5% FBS for 72 h. Cell viability was assessed using the Cell Counting Kit (CCK)-8 assay kit (Dojindo Molecular Technologies, Inc., Japan) according to the manufacturer’s instructions. The absorbance was measured using the Hidex sense beta plus microplate reader (HIDEX, Finland).

**RESULTS**

Dovitinib enhances BMP-2-induced osteoblast differentiation via the Smad1/5/8-mediated signaling pathway in C2C12 cells

First, to determine the optimal concentration of dovitinib (Fig. 1A)
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Fig. 1. The effect of dovitinib on the viability of BMP-2-stimulated C2C12 cells. (A) Chemical structure of dovitinib. (B) The C2C12 cells were treated with BMP-2 (50 ng/ml) alone or combined with dovitinib for 72 h. Cell viability was evaluated using the CCK-8 assay. Detailed experimental procedures are described in the Materials and Methods. All experiments were performed in triplicate.

Fig. 2. Dovitinib stimulates the induction of ALP in BMP-2-induced osteoblast differentiation through activation of the Smad1/5/8-mediated signaling pathway. (A) The effect of dovitinib on BMP-2-induced osteoblast differentiation was detected by visualizing the induction of ALP in C2C12 cells. Scale bars represent 100 μm. (B) The activity of ALP, when cells were treated with BMP-2 alone or combined with dovitinib, was measured by a microplate reader. All experiments were performed in triplicate. (C) The C2C12 cells were treated with BMP-2 (50 ng/ml) alone or combined with dovitinib for 2 days. After 3 days, cells were lysed and fractionated to the cytosol and nuclear portions. The phosphorylation and translocation of Smad1/5/8 into the nucleus were measured by Western blot analysis. Actin was used as a loading control.

Dovitinib stimulates the MAPK signaling pathway in BMP-2-induced osteoblast differentiation of C2C12 cells

Additionally, BMP-2 activates non-Smad signaling such as MAPKs, including ERK1/2, p38, and JNK, in the osteoblast differentiation process (Guicheux et al., 2003; Reilly et al., 2005). Dovitinib enhanced the phosphorylation of ERK1/2 and p38 (Fig. 3A), but JNK were not changed (data not shown). To confirm the relevance between BMP-2-induced osteoblast differentiation and MAPK activation, we detected the ALP activity in the presence of dovitinib and the MEK inhibitor PD98059 and p38 inhibitor SB202190. The number of ALP-positive cells was increased by dovitinib treatment, but PD98059 and SB202190 suppressed the ALP activity in a dose-dependent manner (Figs. 3B and 3C). However, dovitinib only did not show dose-dependent enhancing effect on the phosphorylation of ERK1/2 and p38 (Supplementary Fig. S2B). These results support that dovitinib exerts a stimulating effect on BMP-2-induced osteoblast differentiation of C2C12 cells through the activation of MAPK signaling pathways, as well as the Smad-mediated signaling pathway.

Dovitinib activates mRNA expression of osteoblast differentiation marker genes

During BMP-2-mediated osteoblast differentiation, BMP-2 induces transcription of endogenous BMPs in C2C12 cells. Approximately 20 BMPs of vertebrates originally have osteogenic activity, as well as various other physiological activities (De Biase and Capanna, 2005). Among different BMPs, BMP-2, BMP-4, BMP-5, BMP-6, and BMP-7 are most commonly discovered as osteogenic BMPs. We further investigated whether mRNA expression of these osteogenic BMPs and osteogenic...
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Fig. 3. Dovitinib activates MAPK signaling pathways in BMP-2-induced osteoblast differentiation. The C2C12 cells were treated with BMP-2 (50 ng/ml) ± dovitinib for 10 minutes. (A) The phosphorylation of ERK1/2 and p38 was measured by Western blot analysis. Actin was used as a loading control. (B, C) The C2C12 cells were treated with PD98059 or SB202190 in the presence of BMP-2 (50 ng/ml) and dovitinib (100 nM) for 3 days. The effect of inhibitors on the BMP-2-induced osteoblast differentiation was detected by the induction of ALP activity in C2C12 cells. Experiments were performed in triplicate. Scale bars represent 100 μm.

markers such as ALP, Osteocalcin (OCN), and Runx2 is involved in the osteogenic stimulating effect of dovitinib using real-time PCR analysis. Among osteogenic BMPs, the mRNA expression levels of BMP-4, BMP-7, ALP, and OCN were increased by the combination of BMP-2 and dovitinib in a dose-dependent manner (Fig. 4), but the expression levels of other BMPs and Runx2 were not changed (Supplementary Fig. S3). This result indicated that dovitinib enhances the mRNA transcriptional activity of osteogenic markers BMP-4, BMP-7, ALP, and OCN in the BMP-2-induced osteoblast differentiation process.

DISCUSSION

In this study, we investigated the osteogenic effect of dovitinib in the BMP-2-mediated osteoblast differentiation of C2C12 cells. Dovitinib (TKI-258) was originally developed as a multi-targeted receptor tyrosine kinase (RTK) inhibitor and has a potent inhibitory effect on the activities of the Class III (FLT3/c-Kit), Class IV (FGFR1/3), and Class V (VEGFR1-4) RTKs (Lopes de Menezes et al., 2005; Porta et al., 2015). In the previous reports, dovitinib has been used as an anti-cancer agent undergoing preclinical or clinical trials (Angevin et al., 2013; Eritja et al., 2014; Kim et al., 2011; Milowsky et al., 2014).

Until now, dovitinib showed anti-cancer activity in many types of human cancers, but there were no specific reports against bone disease. Although dovitinib shows wide ranges of IC50 in many types of cancer cells, this study demonstrated that relatively low concentrations of dovitinib could exert a stimulating effect on the BMP-2-induced osteoblast differentiation of C2C12 cells without significant cytotoxicity. As mentioned in previous reports, dovitinib showed anti-cancer activity through inhibition of FGFRs, PDGFRs, and VEGFRs (Angevin et al., 2013; Eritja et al., 2014; Kim et al., 2011; Milowsky et al., 2014). However, in C2C12 cells, BMP-2 increased the proliferation of C2C12 cells and dovitinib did not exert significant cytotoxicity. This result implies that relatively low concentrations of dovitinib could not inhibit the activity of FGFRs or PDGFRs to affect the cell viability of BMP-2-treated C2C12 cells. Although, osteoblast differentiation can be mediated by FGF/FGFR or PDGF/PDGFR signaling, multiple signaling networks including TGF-β/BMP, MAPK, Smad, Akt/mTOR, and Wnt signaling and transcription factors tightly regulate osteogenesis or bone formation (Caverzasio et al., 2013; Guicheux et al., 2003; Hipskind and Bilbe, 1998; Kobayashi et al., 2015; Marie et al., 2012; Rahman et al., 2015). Additionally, among the FGFs, FGF-8 suppresses BMP-2-induced osteoblast differentiation through the inhibition of ERK pathway (Katsuyama et al., 2015). This report also supports our results that dovitinib might act as a FGF-8 inhibitor to activate BMP-2-induced osteoblast differentiation. In this regard, we suggest that dovitinib showed synergistic effect on osteoblast differentiation process of C2C12 cells via BMP-2-induced
MAPK and Smad signaling cascades.

The biopharmaceutical industry has rapidly grown and invested a lot of money for the R&D of new drugs; however, the number of approved new drugs has not kept pace with the increase in R&D spending. To reduce the R&D expenditures and increase the success rate, many companies apply various strategies in drug development. Of these cost-reducing approaches, drug repositioning is a new method to search existing developed drugs (Ashburn and Thor, 2004; Chong and Sullivan, 2007). Drug repositioning allows companies to develop new drugs through changing the scope of the original medical indication, leading to faster processing, while reducing the risks of failure in drug discovery and development. In a previous report, we investigated the potential of the anti-cancer drug CX-4945 as a regulator of osteoblast and osteoclast differentiation (Son et al., 2013). This report provided a possibility that anti-cancer drugs in clinical trials or FDA approved can be used for other indications.

Based on the concept of drug repositioning, we also evaluated the effect of dovitinib on BMP-2-induced osteoblast differentiation. Recent reports provide new information about therapeutic applications of anti-cancer agents for the treatment of another disease or synergistic effects with exiting drugs (Beeharry et al., 2014; Bharadwaj et al., 2015; Hanusova et al., 2015; Pemovska et al., 2015; Song et al., 2015). Published reports mainly report that dovitinib exerts anti-cancer activity through inhibition of FGFRs, PDGFRs, and VEGFRs in various types of cancers (Angevin et al., 2013; Entja et al., 2014; Kim et al., 2011; Milowsky et al., 2014). Targeted therapies, associated with specific types of diseases, have improved in drug discovery and development process. However, increasing resistance rates of tumor cells against targeted chemotherapies induce fail in the development of new drugs. Compared with the previous reports, our study did not show dovitinib induced cytotoxicity and inhibitory activity on the expression of FGFRs or PDGFRs in C2C12 cells. Although the biochemical activity of dovitinib was unpredictable and not consistent with published results, our results represent a novel effect of dovitinib in BMP-2-mediated signaling. Our key findings indicated that BMP-2 induces osteogenic metabolisms in C2C12 cells through activation of Smad1/5/8 and MAPK mediated signaling and the transcriptional activity of osteogenic BMPs, such as BMP-4 and BMP-7. This process of BMP-2-induced osteoblast differentiation was enhanced by combined treatment with dovitinib, without a significant cytotoxic effect.

Bone homeostasis is regulated by the ongoing balance between osteoclastic bone resorption and osteoblastic bone formation (Harada and Rodan, 2003). The physiological imbalance in bone remodeling between the differentiation of osteoblasts and osteoclasts results in the bone mass decrease and skeletal disorders such as osteoporosis (Goltzman, 2002). While most of approved drugs regulating bone metabolism mainly suppresses bone resorption through the inhibition of osteoclast differentiation, development of anabolic drugs that induces osteoblast differentiation is not sufficient yet (Goltzman, 2002). In this regard, our study provides a potent opportunity to identify a new agent with potential activity for bone homeostasis. Since bone homeostasis is regulated by osteoblast differentiation and osteoclast differentiation, we thought that further investigations for the identification of dovitinib activity for bone homeostasis will be needed in future.

Here, we showed the potential for an existing drug through repositioning. This could help reduce the time to discover a novel drug candidate and the optimization of pharmacological characteristics. This study utilized an efficient method for the development of new drugs that has a cost-saving effect on future R&D processes.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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REFERENCES

André, F., Bachelot, T., Campone, M., Delenc, F., Perez-Garcia, J.M., Hurvitz, S.A., Turner, N., Rugo, H., Smith, J.W., Deudon, S. et al. (2013). Targeting FGFR with dovitinib (TKI258): preclinical and clinical data in breast cancer, Clin. Cancer Res. 19, 3693-3702.

Angevin, E., Lopez-Martin, J.A., Lin, C.C., Gschwend, J.E., Harzstark, A., Castelliano, D., Soria, J.C., Sen, P., Chang, J., Shi, M. et al. (2013). Phase I study of dovitinib (TKI258), an oral FGFR, VEGFR, and PDGFR inhibitor, in advanced or metastatic renal cell carcinoma. Clin. Cancer Res. 19, 1257-1268.

Ashburn, T.T., and Thor, K.B. (2004). Drug repositioning: identifying and developing new uses for existing drugs. Nat. Rev. Drug Discov. 3, 673-683.

Beeharry, N., Banina, E., Hittle, J., Skobeleva, N., Khazak, V., Deacon, S., Andrade, M., Eglington, B.L., Peterson, J.R., Astsaturov, I., et al. (2014). Re-purposing clinical kinase inhibitors to enhance chemosensitivity by overriding checkpoints. Cell Cycle 13, 2172-2191.

Bharadwaj, U., Eckols, T.K., Kolosov, M., Kasembeli, M.M., Adam, A., Torres, D., Zhang, X., Dobrolecki, L.E., Wei, W., Lewis, M.T., et al. (2015). Drug-repositioning screening identified piperlonguminine as a direct STAT3 inhibitor with potent activity against cancer. Oncogene 34, 1341-1353.

Boyle, W.J., Simonet, W.S., and Lacey, D.L. (2003). Osteoclast differentiation and activation, Nature 423, 337-342.

Candellere, G.A., Liu, F., and Aubin, J.E. (2001). Individual osteoblasts in the developing calvaria express different gene repertoires, Bone 28, 351-361.

Cao, X., and Chen, D. (2005). The BMP signaling and in vivo bone formation. Gene. 357, 1-8.

Caverzasio, J., Biver, E., and Thouverey, C. (2013). Predominant role of PDGF receptor transactivation in Wnt5a-induced osteoblastic cell proliferation. J. Bone Miner. Res. 28, 260-270.

Chae, H.J., Jeong, B.J., Ha, M.S., Lee, J.K., Byun, J.O., Jung, W.Y., Yun, Y.G., Lee, D.G., Oh, S.H., and Chae, S.W., et al. (2002). ERK MAP kinase is required in 1,25(OH)2D3-induced differentiation in human osteoblasts, Immunopharmacol. Immunotoxicol. 24, 31-41.

Chong, C.R., and Sullivan, D.J. Jr. (2007). New uses for old drugs. Nature 448, 645-646.

De Blase, P., and Capanna, R. (2005). Clinical applications of BMPs. Injury 36, S43-46.

Entja, N., Domingo, M., Dosil, M.A., Mirantes, C., Santacana, M., Valles, J., Llobert-Cussac, A., Matias-Guiu, X., and Dolcet, X. (2014). Combinatorial therapy using dovitinib and IC182,780 (fulvestrant) blocks tumoral activity of endometrial cancer cells. Mol. Cancer Ther. 13, 776-787.

Franceschi, R.T., and Iyer, B.S. (1992). Relationship between collagen synthesis and expression of the osteoblast phenotype in MC3T3-E1 cells. J. Bone Miner. Res. 7, 235-246.

Garces, C., and Garcia, L.E. (2006). Combination of anabolic and antiresorptive agents for the treatment of osteoporosis, Maturitas 54, 47-54.

Garrett, I.R. (2007). Anabolic agents and the bone morphogenetic...
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protein pathway, Curr. Top. Dev. Biol. 78, 127-171.
Goltzman, D. (2002). Discoveries, drugs and skeletal disorders. Nat. Rev. Drug Discov. 1, 784-796.
Guichaux, J., Lemonnier, J., Ghayor, C., Suzuki, A., Palmer, G., Caverzasio, J. (2003). Activation of p38 mitogen-activated protein kinase and c-Jun-NH2-terminal kinase by BMP-2 and their implication in the stimulation of osteoblastic cell differentiation. J. Bone Miner. Res. 18, 2060-2069.
Hanusova, V., Skalova, L., Kralova, V., and Matouskova, P. (2015). Potential anti-cancer drugs commonly used for other indications. Curr. Cancer Drug Targets 15, 35-52.
Harada, S. and Rodan, G.A. (2003). Control of osteoblast function and regulation of bone mass. Nature 423, 349-355.
Hasinoff, B.B., Wu, X., Nitiss, J.L., Kanagasabai, R., and Yalowich, J.C. (2012). The anticancer multi-kinase inhibitor dovitinib also targets topoisomerase I and topoisomerase II, Biochem. Pharmacol. 84, 1617-1626.
Hipskind, R.A. and Bilbe, G. (1998). MAP kinase signaling cascades gene expression in osteoblasts. Front Biosci. 3, d804-816. S. Katagiri, T., Yamaguchi A., Komaki, M., Abe, E., Takahashi, N., Ikeda, T., Rosen, V., Wozney, J.M., Fujisawa-Sehara, A., and Suda, T. (1994). Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage, J. Cell Biol. 127, 1755-1766.
Katsuyama, T., Otuka, F., Terasaka, T., Inagaki, K., Takano-Narazaki, M., Matsumoto, Y., Sada, K.E., and Makino, H. (2015). Regulation of effects of fibroblast growth factor-8 and tumor necrosis factor-α on osteoblast marker expression induced by bone morphogenetic protein-2. Peptides 73, 88-94.
Kim, K.B., Chesney, J., Robinson, D., Gardner, H., Shi, M.M., and Stuart, M. (2004). Rediscovering existing drugs, Start-Up Successes, In Vivo 18, 127-171.
Lee, S.H., Lopes de Menezes, D., Vora, J. Harris, A., Ye, H., Nor- dahl, L., Garrett, E., Samara, E., Aukerman, S.L., Gelb, A.B., and Heise, C. (2005). In vivo target modulation and biological activity of CHIR-258, a multитargeted growth factor receptor kinase inhibitor, in colon cancer models, Clin. Cancer Res. 11, 3633-3641.
Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta Ct()) Method. Methods 25, 402-408.
Long, F. (2012). Building strong bones: molecular regulation of the osteoblast lineage, Nat. Rev. Mol. Cell Biol. 13, 27-38.
Longman, R. (2004). Pharmaceutical strategies: jumpstart to products, In Vivo 18, 22, 17.
Lopes de Menezes, D.E., Peng, J., Garrett, E.N., Louie, S.G., Lee, S.H., Wiesmann, M., Tang, Y., Shephard, L., Goldbeck, C., Oei, et al. (2005). CHIR-258: a potent inhibitor of FLT3 kinase in experimental tumor xenograft models of human acute myelogenous leukemia, Clin. Cancer Res. 11, 5281-5291.
Marie, P.J., Miraoui, H., and Sèvère, N. (2012). FGFR/FGFR signaling in bone formation: progress and perspectives, Growth Factors 30, 117-123.
Mihowsky, M.I., Dittrich, C., Durán, I., Jagdev, S., Millard, F.E., Sweeney, C.J., Bajorin, D., Cerbone, L., Quinn, D.I., Studlar, et al. (2014). Phase 2 trial of dovitinib in patients with progressive FGFR3-mutated or FGFR3 wild-type advanced urothelial carcinoma, Eur. J. Cancer 50, 3145-3152.
Pemovska, T., Johnson, E., Kontro, M., Repasky, G.A., Chen, J., Wells, P., Cronin, C.N., McTigue, M., Kallioniemi, O., PORKKA, K., et al. (2015). Axitinib effectively inhibits BCR-ABL1(T315I) with a kinase binding conformation. Nature 519, 102-105.
Phimphliai, M., Zhao, Z., Boules, H., Roca, H., and Franceschi, R.T. (2006). BMP signaling is required for RUNX2-dependent induction of the osteoblast phenotype, J. Bone Miner. Res. 21, 637-646.
Porta, C., Guglione, P., Liguiji, W., and Paglino, C. (2015). Dovitinib (Giotrius, TKI258): structure, development and preclinical and clinical activity. Future Oncol. 11, 39-50.
Rahman, M.S., Akhtar, N., Jamil, H.M., Banik, R.S., and Asaduzzaman, S.M. (2015). TGF-β/BMP signaling and other molecular events: regulation of osteoblastogenesis and bone formation. Bone Res. 3, 15005.
Reilly, G.C., Golden, E.B., Grasso-Knight, G., and Leboy, P.S. (2005). Differential effects of ERK and p38 signaling in BMP-2 stimulated hypertrophy of cultured chick sternal chondrocytes. Cell Commun. Signal. 3, 3.
Rosen, V. (2009). BMP signaling in bone development and repair, Cytokine Growth Factor Rev. 20, 475-480.
Rosen, C.J., and Bilezikjian, J.P. (2001). Clinical review 123: ana
c
tolic therapy for osteoprosis, J. Clin. Endocrinol. Metab. 86, 957-964.
Sarker, D., Mollie, R., Evans, T.R., Hardie, M., Marriott, C., Butzberger-Zimmerli, P., Morrison, R., Fox, J.A., Heise, C., Louie, S., et al. (2008). A phase I pharmacokinetic and pharmacodynamic study of TK258, an oral, multitargeted receptor tyrosine kinase inhibitor in patients with advanced solid tumors, Clin. Cancer Res. 14, 2075-2081.
Son, Y.H., Moon, S.H., and Kim, J. (2013). The protein kinase 2 inhibitor CX-4945 regulates osteoclast and osteoblast differentiation in vitro, Mol. Cells 36, 417-423.
Song, M., Kim, S.H., and Yoon, S.K. (2015). Cabozantinib for the treatment of non-small cell lung cancer with KIT5F-BRET fusion. An example of swift reppositioning, Arch. Pharm. Res. 38, 2120-2123.
Stuart, M. (2004). Rediscovering existing drugs, Start-Up 9, 23-30.
Suzukim, A., Guichaux, J., Palmer, G., Miura, Y., Oiso, Y., Bonjour, J., and Caverzasio, J.P. (2002). Evidence for a role of p38 MAP kinase in expression of alkaline phosphatase during osteoblastic cell differentiation, Bone 30, 91-98.
Trudel, S., Li, Z.H., Wei, E., Wiesmann, M., Chang, H., Chen, C., Reese, D., Heise, C., and Stewart, A.K. (2006). CHIR-258, a novel, multitargeted tyrosine kinase inhibitor for the potential treatment of t(4;14) multiple myeloma, Blood 105, 2941-2948.
Wagner, D.O., Sieber, C., Bhushan, R., Borgermann, J.H., Grat, D., and Kraus, P. (2010). BMPs: from bone to body morphogenetic proteins, Sci. Signal 3, mr1.
Wang, X., Kay, A., Anak, O., Angevin, E., Escudier, B., Zhou, W., Feng, Y., Dugan, H., and Schran, M. (2013). Population pharma
cokinetic/pharmacodynamic modeling to assist dosing schedule selection for dovitinib, J. Clin. Pharmacol. 53, 14-20.
Wu, C.C., Li, Y.S., Haga, J.H., Wang, N., Lian, I.Y., Su, F.C., Usamim, S., and Chien, S. (2006). Roles of MAP kinases in the regulation of bone matrix gene expressions in human osteoblasts by oscillatory fluid flow, J. Cell. Biochem. 98, 632-641.