The role of Rho GTPases substrates Rac and Cdc42 in osteoclastogenesis and relevant natural medicinal products study

Yuan Liu¹,²,#, Yusheng Dou³, Liang Yan¹, Xiaobin Yang¹, Baorong He¹, *, Lingbo Kong¹,*, Wanli Smith

1. Department of Spine Surgery, Honghui Hospital, School of Medicine, Xi’an Jiaotong University. China
2. Yan’an University Medical School, Yan’an, China;
3. Department of Shoulder and Elbow Joint, Honghui Hospital, School of Medicine, Xi’an Jiaotong University. China
4. Department of Psychiatry and Behavioral Sciences, School of Medicine, Johns Hopkins University, Baltimore, Maryland, USA

*: Corresponding to 1. Lingbo Kong: lingbokong@163.com; and 2. Baorong He: baoronghespine@163.com; Honghui-hospital, xi’an Jiaotong univeristy, school of medicine, Xi’an China

Abstract:
Recently, Rho GTPases substrates include Rac (Rac1 and Rac2) and Cdc42 have been reported to exert multiple cellular functions in osteoclasts, the most prominent of which includes regulating the dynamic actin cytoskeleton rearrangements. In addition, natural products and their molecular frameworks have a long tradition as valuable starting points for medicinal chemistry and drug discovery. Although currently there has no report about the natural product, which could play a therapeutic role on bone loss diseases (osteoporosis and osteolysis) that through the regulation of Rac1/2 and Cdc42 during osteoclasts cytoskeletal structuring, there have several excellent studies for exploring the therapeutic potentials of various natural products for their role in inhibit cancer cells migration and function via regulating the Rac1/2 and Cdc42. Herein in this review, we try to focus on recent advances studies for extensively understanding the role of Rho GTPases substrates Rac1, Rac2 and Cdc42 in osteoclastogenesis, as well as therapeutic potentials of natural medicinal products for their properties on the regulation of Rac1, and/or Rac2 and Cdc42, which in order to inspire drug discovery in regulating osteoclastogenesis.
Keywords: Rho GTPases, Rac1, Rac2, Cdc42, Osteoclast, Bone, Natural Compounds,

Introduction

Osteoclastogenesis has been defined as a multi-step processes of osteoclast differentiation [1], which including several osteoclastic cellular biological functions; such as: migration, cellular contact, cellular fusion and cellular responding to extracellular factors[2]. Document studies demonstrated that osteoclastogenesis initially mediated by two critical cytokines, the macrophage colony stimulating factor-1 (M-CSF) and the receptor activator of nuclear factor-kappaB ligand (RANKL)[3]. In that, M-CSF binds to its receptor (cFms) present in osteoclast precursors, which stimulates their proliferation and inhibits their apoptosis. While, RANKL interact with its receptor RANK in osteoclast precursor cells, osteoclastogenesis is induced[4] (Figure 1).

However, at the late stage of osteoclastogenesis, osteoclastic polarization characterized the final maturation of bone resorptive osteoclasts. Notably, during the bone resorption process, osteoclastic polarization involves rearrangement of the actin cytoskeleton, in which an filamentous (F)-actin ring that comprises a dense continuous zones of highly dynamic podosomes are formed and consequently an area of membrane that develop into the ruffled border is isolated[5, 6].
**Cytoskeletal rearrangement during osteoclastogenesis**

It is worthy to noted that during the cytoskeletal rearrangement among the osteoclastogenesis, podosome is the most prominent cytoskeletal structure for the degradation of mineralized bone matrix and associate to the mobility of osteoclast[7]. In fact, podosome is not exclusive organelle in osteoclast, which also include endothelial cells, and cells from the monocytic lineage such as: dendritic cells (DCs) and macrophages[8]. Regardless the presenting of podosomes in various cells, podosomes patterning play a crucial and unique role for the support osteoclast final maturation[8]. As early as individual podosome form within an osteoclast, they are collectively and sequentially organized into different patterns along the life of the same cell. However, these patterns evolve along with osteoclastogenesis from monocytes/macrophages to osteoclast precursors, further to the bone resorptive matured osteoclast. In the early stage of osteoclastogenesis, podosome pattern from apparently random groups of “clusters” to circle pattern “rings” in the middle term stage[9]. Eventually, in the late stage of osteoclastogenesis, podosome pattern into much massive circular structures, i.e., either “sealing zone like structures” (SZL, also known as “belts”) or “sealing zones” (SZ) [10].

**Rac isoforms** *(Rac1 and Rac2)* **in regulation of cytoskeletal**
It has been reported that Rac1 and Rac2 are critical GTPase to osteoclast formation and maturation. In fact, Rac1 and Rac2, are intimately associated with the organization of the different types of cellular cytoskeleton, such as: osteoclast, DCs and macrophages. Notably, these two isoforms are also involved in the osteoclastic adhesive function formation and subsequent bone resorption[11, 12]. However, the specific role of Rac1 and Rac2 in osteoclastogenesis still unknown. For example, osteoclasts contain NADPH diaphorase activity[13, 14], and free radicals which both could influence bone resorption, however, Rac1 and Rac2 are also essential components of NADPH oxidase[15-18], the enzyme responsible for generating free radicals. Besides that, study has also demonstrated Rac1 and Rac2 could through regulating the generation of reactive oxygen species (ROS)[19] and actin remodeling participating the osteoclastogenesis regulation. Recent study has found that both Rac1 and Rac2 are required for normal RANKL induced osteoclast differentiation, but Rac1 deletion results in a more profound reduction in osteoclast formation in vitro because of its regulatory role in pre-osteoclast M-CSF mediated chemotaxis and actin assembly and RANKL-mediated reactive oxygen species generation[20]. These results speculated that Rac1 and Rac2 might function osteoclastic organelle actin dynamics regulating, such as: actin filament ends and podosomes. In fact, Rac1 and Rac2
proteins have overlapping roles in podosome assembly and SZL formation by localizing Arp2/3 at podosome sites during osteoclastogenesis[7, 21-27]. Osteoclasts generated from the Rac1 and Rac2 double knockout mouse are devoid of podosomes and SZ, which finally showed impaired bone resorption capacities[24, 28-32]. Notably, however, these defects are observed only if Rac1 and Rac2 deletion occurs at the early osteoclast precursor stage, which means the Rac1 and Rac2 deficient osteoclasts lack the capabilities on actin cytoskeletal formation.

**The role of Cdc42 in regulation the podosome of osteoclast**

Cdc42 is another Rho family small GTPase[33]. As a downstream signaling of RANKL, Cdc42 might interact with the Crib domain of the adaptor Par3[34, 35], Par6 and atypical PKC (aPKC)[36-38], which forming a quaternary complex to cascade the upper signaling transduction from RANKL and RANK binding, further stimulate the osteoclastogenesis. However, unlike Rac1 and Rac2 the definition role of Cdc42 in osteoclastogenesis is much clearly associate with its actin regulative effects, i.e the podosome regulation. Recent studies using mice with increased Cdc42 activation due to knockout of its negative regulator Cdc42GAP have shown increased sealing zone formation and bone resorption, compared to wildtype cells[27, 39].
Cdc42 stands as a central player in the regulation of podosome dynamics as it orchestrates podosome actin polymerization, which from the monomeric globular (G)-actin into filamentous (F)-actin, through its canonical effector, Wiscott Aldrich Syndrome protein (WASp)[40-42]. WASp depletion in macrophages leads to a virtual absence of podosomes and a defective chemotactic response under a gradient of M-CSF. Cdc42 binds directly to WASp, a multi-domain adapter protein regulating transmission of signals to the actin cytoskeleton. This binding, together with phosphorylation of WASp on tyrosine, induces a dramatic conformational change[40, 41, 43]. The hydrophobic core is disrupted, releasing the VCA (Verprolin Homology domain-cofilin homology domain–Acidic region) domain and enabling its interaction with the Arp2/3 complex, thereby promoting actin nucleation[44-46].

**Natural products that targeting on the regulation of Rac1&Rac2, and Cdc42**

Natural products and their molecular frameworks have a long tradition as valuable starting points for medicinal chemistry and drug discovery. Recently, there has been a revitalization of interest in the inclusion of these chemotypes in compound collections for screening and achieving selective target modulation. Although currently there has no report on the
natural product, which could play a therapeutic role on bone loss diseases (osteoporosis and osteolysis) that through the regulation of Rac1/2 and Cdc42 during osteoclasts cytoskeletal structuring, there have several excellent studies for exploring the therapeutic potentials of various natural products in regulating cancer cells migration and function (Table 1). Here we collected several natural products with a focus on recent advances in their properties on the regulation of Rac1, and/or Rac2 and Cdc42, and related signaling molecular, which in order to inspire drug discovery in regulating osteoclastogenesis (Figure 2).

**Rac1&Rac2 regulative natural products**

*Fisetin* (3,3′,4′,7-Tetrahydroxyflavone), is natural product that could be found in vegetable and fruits[47]. *Fisetin* has been well established possesses anti-oxidant[48], and anti-neurodegenerative progression[49]. Most recently, Jacob et al.[50] have reported *Fisetin* showed a significant protective effect on developmental Methyl mercury neurotoxicity in the F1 generation of MeHg exposed rats. In that, Methyl mercury is a teratogenic and neurodevelopmental toxicant in the environment. Whereas, MeHg could affect several biological pathways critical for brain development. Most recently, authors present study validated the effect of *Fisetin* on developmental MeHg exposure induced alterations in mitochondrial apoptotic pathway and Rho GTPase mRNA expressions in
hippocampus of F1 generation rats. Their extensively study showed that Fisetin against gestational MeHg exposure induced changes in expression of ERK / Caspase 3 genes of apoptosis signalling pathway and Rho A/ Rac1/Cdc42 genes of Rho GTPase signaling pathway in hippocampus of F1 generation weaning Wistar rats.

*Deacetyl-mycoepoxydiene* (DA-MED) is a 248 molecular weight compound that has been isolated from the endophytic fungus, *Phomopsis sp.*, of costal mangrove plants and has been shown to be a secondary metabolite with a rare oxygen-bridged cyclopentadiene skeleton[51]. This compound has cytotoxic activities toward various cell lines, including A549, HCC-S102 and HepG2 cells with IC50 values ranging from 1.05–1.95 mg/mL. Recently, Xie et al.[52] have reported that DA-MED treatment drives Rac1 activation and promotes robust production of reactive oxygen species, activating mitochondrial permeability transition and the intrinsic apoptotic pathway. Knockdown of Rac1 decreases ROS production in DA-MED-treated cells, resulting in a concomitant decrease in DA-MED-induced apoptosis. DA-MED-activated Rac1 induces autophagy by inhibiting mammalian target of rapamycin, leading to anti-apoptotic and anti-metastatic effects. Therefore, this study provides novel insight into the complex cytotoxic and pro-survival mechanisms associated with a potent Rac1 agonist and suggests that further
development of more potent Rac1 agonists could be an effective strategy for future non-small cell lung cancer treatments.

*Diallyl disulfide* (DADS), one of the sulfur compounds derived from garlic, exhibits biological activity via modulating molecules and signaling pathways in various cell physiologies[53-57]. These properties suggesting that DADS could be used as a potential therapeutic compound for the treatment or prevention of various diseases. Moreover, study has demonstrated that transforming growth factor-β1 (TGF-β1) could promote epithelial-mesenchymal transition (EMT), invasion and proliferation through the activation of Rac1 and β-catenin signaling pathways. Therefore, Su B et al.[55] have conducted a study for investigating the effects of DADS on TGF-β1-induced EMT and cellular invasion. Primarily, they found TGF-β1 treatment augmented EMT and invasion, concomitantly with increased expression of Rac1 and β-catenin. However, the DADS treatment could decrease the activities of Rac1 and β-catenin. DADS, TGF-β1 receptor inhibitor as well as Rac1 inhibitor antagonized the upregulation of the TGF-β1-induced expression of these genes, abolishing the enhanced effects of TGF-β1 on EMT and invasion. These data indicated that the blockage of TGF-β1/Rac1 signaling by DADS may be responsible for the suppression of EMT and cellular invasion.
Mulberry (M. alba L.) is a common fruit in temperate, subtropical, and tropical areas, and contains abundant polyphenols and anthocyanin components[58, 59]. Study showed the anthocyanins from the mulberry could inhibit the B16-F1 cell lineage invasion[60]. The underlying molecular mechanisms is anthocyanins partly suppressed the Ras/PI3K signaling pathway. In addition, mulberry polyphenol extract (MPE) is rich in polyphenols that have antioxidant, anti-inflammatory, anti-aging, anti-obesity, and anti-tumor effects. Considering the biological effects of anthocyanins, further study performed by Yu et al.[58] investigated that MPE on treating vascular smooth muscle cellular migration and proliferation. Their results showed that MPE could suppress the expression of FAK, Src, PI3K, Akt, c-Raf, and inhibit the signaling axis of FAK/Src/PI3K in cell. Besides that, their study also showed that MPE decrease the expression of small Rac1 and Cdc42 to affect F-actin cytoskeleton rearrangement.

As aforementioned, cytoskeletal structure rearrangement grant various cellular functions in various cell linages, such as: podosome patterning in osteoclast, and epithelial-to-mesenchymal transition. This has led to a surge in the efforts for identification of safer and more effective compounds which can modulate these cellular behaviors. Plectranthoic


acid (PA), a natural compound isolated from the extracts of *Ficus microcarpa*, has been reported possesses the capability to induce cell cycle arrest and apoptosis in prostate cancer cells[61, 62]. Recently, Akhtar et al.[61] extensively studied the PA biological effects on suppressing the cellular migration. Through the proteomic analysis authors identified Rac1 is the major cadherin signaling protein modulated with PA treatment.

**Cdc42 regulative natural products**

*Cudrania cochinchinensis* (Moraceae) has been reported for its potent biological activites such as: anti-inflammation[63] and neuroprotective effects[64]. Whereas, the compound *Cudraxanthone-S* derived from *Cudrania cochinchinensis* was studied for its pharmacokinetics and binding potential in treating the fungal infection of Candida albicans, which could cause several lethal infections in immune-suppressed patients and recently emerged as drug-resistant pathogens worldwide[65]. Authors found *Cudraxanthone-S* were exhibited ability on regulating the Cdc42 in MAPK signaling pathway.

*Panacis Japonici Rhizoma* (PJR), derived from dry rhizome of *Panax japonicus C. A. Meyer* (Araliaceae), distributes in the southwest of China[66-68]. As a widely used focal medicine, the PJR manifested
similar clinical merits in anti-tussive and anti-inflammatory diseases[69, 70]. Recently, Chen et al.[71] have demonstrated that PJR could suppress the HEY and A2780 cells migration and invasion by decrease the Cdc42 and Rac activity.

Triptolide (TP), derived from the medicinal plant *Triterygium wilfordii Hook. f.* (TWHF), is a diterpene triepoxide with variety biological and pharmacological activities[72]. Wang et al.[73] has studied the cytoskeletal structuring effects of TP on Sertoli cells, which play a critical role during spermatogenesis. Their study results demonstrate that TP can regulating the Sertoli cellular cytoskeleton structuring via inhibit the expression of Cdc42.

The compound (4,5,4'-trihydroxy-3,3'-dimethoxybibenzyl (TDB)) extract from *Dendrobium ellipsophyllum Tang and Wang*, has been demonstrated to have antime metastatic activity through the sensitization of detachment-induced cell death[74, 75]. Study from Chaotham et al.[76] showing that TDB reduced such cell migration and invasion by decreasing migration-regulating proteins, including integrins αv, α4, β1, β3 and β5, as well as downstream signaling proteins, such as activated focal adhesion kinase (pFAK), activated Rac1 and Cdc42. As the presence of cellular protrusion, called filopodia, has been indicated as a
hallmark of migrating cells, we showed that the reduction of the mentioned proteins correlated well with the disappearance of filopodia. In summary, this study demonstrates the promising activity of TDB and its mechanism in the inhibition of lung cancer cell migration, which might be useful for encouraging the development of this compound for antimetastatic approaches.

Inhibition of VEGFR2 activity has been proposed as an important strategy for the clinical treatment of hepatocellular carcinoma (HCC). Corosolic acid (CA), which exists in the root of Actinidia chinensis, as having a significant anti-cancer effect on HCC cells by decreasing the tumor cellular migration. Ku et al. [77] have extensively studied the effects of CA on its cellular regulating effects found that CA inhibits VEGFR2 kinase activity by directly interacting with the ATP binding pocket. Moreover, they found CA could decrease the VEGFR2/Src/FAK/Cdc42 axis, subsequently decreasing F-actin formation and migratory activity in vitro.

Gigantol is a bibenzyl compound derived from the Thai orchid, Dendrobium draconis. It exhibits significant cytotoxic activity against several cancer cell lines. Study conducted by Charoenerunruang et al.[78] demonstrates that gigantol suppresses the migratory cellular behavior via
decreasing Cdc42, thereby suppressing filopodia formation. The inhibitory activity of *Gigantol* on lung cancer cellular migration suggests that this compound may be suitable for further development for the treatment of osteoclastogenesis by regulating the osteoclastic cytoskeletal structuring.

**Conclusion**

Characterized by the unique property, osteoclasts have been extensively studied for its differentiation and cellular functions during the bone homeostasis and pathological process, which make it as a critical target for therapy the bone loss diseases, such as: osteoporosis and osteolysis. Given that the low production costs and the increasing evidence of the ability to target the cellular activities and signaling cascades relevant to various diseases, naturally occurring compounds have received extensive attention as potential therapeutic osteoclastogenesis. Our current review has outlined some naturally occurring compounds, which have shown merit in terms of regulating macrophage polarization. However, given that current natural compounds have the Rac and Cdc42 regulatory effects on cancer cell line, the specific mechanisms and therapeutic effects on osteoclastogenesis remain incompletely understood. Clearly, more in-depth characterization of osteoclast cytoskeleton rearrangement and relevant therapeutic compounds should be conducted to identify the
best possible strategies.

**Declarations**

**Consent for publication**

The manuscript is approved by all authors for publication.

**Availability of data and materials**

All data and materials were included in the manuscript.

**Competing Interests**

The authors declare that they have no competing interests.

**Authors Contribution**

Yuan Liu, Yusheng Dou, Baorong He, Lingbo Kong: conception and design, drawing and interpretation of data; draft the manuscript and revise it critically for important intellectual content; final approval of the version to be published. Liang Yan, Xiaobin Yang: acquisition of data (manuscript reviewing), analysis and interpretation of data. Wanli Smith: design, revise the manuscript critically for important intellectual content. All authors final approval of the version to be published.

**Funding**
This study was supported by a grant from China Postdoctoral Science Foundation, PR.China (2018T111085); Shaanxi provincial science and technology department, science and technology achievement transfer and promotion plan award achievement transformation project, P.R.China (2018HJCG-08); Xi'an science and technology bureau, social development public project (2017115 SF/YX009)

Acknowledgements

N/A

References:

1. Feng W, Xia W, Ye Q, Wu W: Osteoclastogenesis and osteoimmunology. *Front Biosci (Landmark Ed)* 2014, 19:758-767.

2. Kukita T, Kukita A, Watanabe T, Iijima T: Osteoclast differentiation antigen, distinct from receptor activator of nuclear factor kappa B, is involved in osteoclastogenesis under calcitonin-regulated conditions. *J Endocrinol* 2001, 170(1):175-183.

3. Baud'huin M, Lamoureux F, Duplomb L, Redini F, Heymann D: RANKL, RANK, osteoprotegerin: key partners of osteoimmunology and vascular diseases. *Cell Mol Life Sci* 2007, 64(18):2334-2350.

4. Crotti TN, Dharmapatni AA, Alias E, Haynes DR: Osteoimmunology: Major and Costimulatory Pathway Expression Associated with Chronic Inflammatory Induced Bone Loss. *J Immunol Res* 2015, 2015:281287.
5. Furlan F, Galbiati C, Jorgensen NR, Jensen JE, Mrak E, Rubinacci A, Talotta F, Verde P, Blasi F: Urokinase plasminogen activator receptor affects bone homeostasis by regulating osteoblast and osteoclast function. *J Bone Miner Res* 2007, **22**(9):1387-1396.

6. Roscher A, Hasegawa T, Dohnke S, Ocana-Morgner C, Amizuka N, Jessberger R, Garbe AI: The F-actin modulator SWAP-70 controls podosome patterning in osteoclasts. *Bone Rep* 2016, **5**:214-221.

7. Georgess D, Machuca-Gayet I, Blangy A, Jurdic P: Podosome organization drives osteoclast-mediated bone resorption. *Cell Adh Migr* 2014, **8**(3):191-204.

8. Hu S, Planus E, Georgess D, Place C, Wang X, Albigez-Rizo C, Jurdic P, Geminard JC: Podosome rings generate forces that drive saltatory osteoclast migration. *Mol Biol Cell* 2011, **22**(17):3120-3126.

9. Luxenburg C, Addadi L, Geiger B: The molecular dynamics of osteoclast adhesions. *Eur J Cell Biol* 2006, **85**(3-4):203-211.

10. Jurdic P, Saltel F, Chabadel A, Destaing O: Podosome and sealing zone: specificity of the osteoclast model. *Eur J Cell Biol* 2006, **85**(3-4):195-202.

11. Croke M, Ross FP, Korhonen M, Williams DA, Zou W, Teitelbaum SL: Rac deletion in osteoclasts causes severe osteopetrosis. *J Cell Sci* 2011, **124**(Pt 22):3811-3821.

12. Razzouk S, Lieberherr M, Cournot G: Rac-GTPase, osteoclast cytoskeleton and bone resorption. *Eur J Cell Biol* 1999, **78**(4):249-255.

13. Darden AG, Ries WL, Wolf WC, Rodriguiz RM, Key LL, Jr.: Osteoclastic superoxide production and bone resorption: stimulation and inhibition by modulators of NADPH
oxidase. *J Bone Miner Res* 1996, **11**(5):671-675.

14. Goettch C, Babelova A, Trummer O, Erben RG, Rauner M, Rammelt S, Weissmann N, Weinberger V, Benkhoff S, Kampschulte M *et al.*: NADPH oxidase 4 limits bone mass by promoting osteoclastogenesis. *J Clin Invest* 2013, **123**(11):4731-4738.

15. Bokoch GM: *Regulation of innate immunity by Rho GTPases*. *Trends Cell Biol* 2005, **15**(3):163-171.

16. Kwong CH, Adams AG, Leto TL: Characterization of the effector-specifying domain of Rac involved in NADPH oxidase activation. *J Biol Chem* 1995, **270**(34):19868-19872.

17. Lacy P, Mahmudi-Azer S, Bablitz B, Gilchrist M, Fitzharris P, Cheng D, Man SF, Bokoch GM, Moqbel R: Expression and translocation of Rac2 in eosinophils during superoxide generation. *Immunology* 1999, **98**(2):244-252.

18. Zhao X, Carnevale KA, Cathcart MK: Human monocytes use Rac1, not Rac2, in the NADPH oxidase complex. *J Biol Chem* 2003, **278**(42):40788-40792.

19. Lee NK, Choi YG, Baik JY, Han SY, Jeong DW, Bae YS, Kim N, Lee SY: A crucial role for reactive oxygen species in RANKL-induced osteoclast differentiation. *Blood* 2005, **106**(3):852-859.

20. Wang Y, Lebowitz D, Sun C, Thang H, Grynpas MD, Glogauer M: Identifying the relative contributions of Rac1 and Rac2 to osteoclastogenesis. *J Bone Miner Res* 2008, **23**(2):260-270.

21. Shin B, Kupferman J, Schmidt E, Polleux F, Delany AM, Lee SK: *Rac1 Inhibition Via Srgap2 Restraints Inflammatory Osteoclastogenesis and Limits the Clastokine, SLIT3*. *J Bone Miner Res* 2019.
22. Guimbal S, Morel A, Guerit D, Chardon M, Blangy A, Vives V: Dock5 is a new regulator of microtubule dynamic instability in osteoclasts. *Biol Cell* 2019, 111(11):271-283.

23. Soares MPR, Silva DP, Uehara IA, Ramos ES, Jr., Alabarse PVG, Fukada SY, da Luz FC, Vieira LO, Oliveira APL, Silva MJB: The use of apocynin inhibits osteoclastogenesis. *Cell Biol Int* 2019, 43(5):466-475.

24. Xu S, Zhang Y, Wang J, Li K, Tan K, Liang K, Shen J, Cai D, Jin D, Li M et al: TSC1 regulates osteoclast podosome organization and bone resorption through mTORC1 and Rac1/Cdc42. *Cell Death Differ* 2018, 25(9):1549-1566.

25. Liu Y, Wang Z, Ma C, Wei Z, Chen K, Wang C, Zhou C, Chen L, Zhang Q, Chen Z et al: Dracorhodin perchlorate inhibits osteoclastogenesis through repressing RANKL-stimulated NFATc1 activity. *J Cell Mol Med* 2020.

26. Tasca A, Astleford K, Lederman A, Jensen ED, Lee BS, Gopalakrishnan R, Mansky KC: Regulation of Osteoclast Differentiation by Myosin X. *Sci Rep* 2017, 7(1):7603.

27. Itzstein C, Coxon FP, Rogers MJ: The regulation of osteoclast function and bone resorption by small GTPases. *Small GTPases* 2011, 2(3):117-130.

28. Gerasimcik N, Westerberg LS, Severinson E: Methods to Study the Role of Cdc42, Rac1, and Rac2 in B-Cell Cytoskeletal Responses. *Methods Mol Biol* 2018, 1821:235-246.

29. Kotelevets L, Walker F, Mamadou G, Lehy T, Jordan P, Chastre E: The Rac1 splice form Rac1b favors mouse colonic mucosa regeneration and contributes to intestinal cancer progression. *Oncogene* 2018, 37(46):6054-6068.
30. Lin J, Lee D, Choi Y, Lee SY: The scaffold protein RACK1 mediates the RANKL-dependent activation of p38 MAPK in osteoclast precursors. Sci Signal 2015, 8(379):ra54.

31. Liu L, Li J, Zhang L, Zhang F, Zhang R, Chen X, Brakebusch C, Wang Z, Liu X: Cofilin phosphorylation is elevated after F-actin disassembly induced by Rac1 depletion. Biofactors 2015, 41(5):352-359.

32. Joshi S, Singh AR, Zulcic M, Bao L, Messer K, Ideker T, Dutkowski J, Durden DL: Rac2 controls tumor growth, metastasis and M1-M2 macrophage differentiation in vivo. PLoS One 2014, 9(4):e95893.

33. Ahmed S, Prigmore E, Govind S, Veryard C, Kozma R, Wientjes FB, Segal AW, Lim L: Cryptic Rac-binding and p21(Cdc42Hs/Rac)-activated kinase phosphorylation sites of NADPH oxidase component p67(phox). J Biol Chem 1998, 273(25):15693-15701.

34. Manser E, Chong C, Zhao ZS, Leung T, Michael G, Hall C, Lim L: Molecular cloning of a new member of the p21-Cdc42/Rac-activated kinase (PAK) family. J Biol Chem 1995, 270(42):25070-25078.

35. Meriane M, Mary S, Comunale F, Vignal E, Fort P, Gauthier-Rouviere C: Cdc42Hs and Rac1 GTPases induce the collapse of the vimentin intermediate filament network. J Biol Chem 2000, 275(42):33046-33052.

36. Ito Y, Teitelbaum SL, Zou W, Zheng Y, Johnson JF, Chappel J, Ross FP, Zhao H: Cdc42 regulates bone modeling and remodeling in mice by modulating RANKL/M-CSF signaling and osteoclast polarization. J Clin Invest 2010, 120(6):1981-1993.
37. Kim H, Choi HK, Shin JH, Kim KH, Huh JY, Lee SA, Ko CY, Kim HS, Shin HI, Lee HJ et al: Selective inhibition of RANK blocks osteoclast maturation and function and prevents bone loss in mice. *J Clin Invest* 2009, 119(4):813-825.

38. Mediero A, Perez-Aso M, Cronstein BN: Activation of EPAC1/2 is essential for osteoclast formation by modulating NFκB nuclear translocation and actin cytoskeleton rearrangements. *FASEB J* 2014, 28(11):4901-4913.

39. Touaitahuata H, Blangy A, Vives V: Modulation of osteoclast differentiation and bone resorption by Rho GTPases. *Small GTPases* 2014, 5:28119.

40. Amato C, Thomason PA, Davidson AJ, Swaminathan K, Ismail S, Machesky LM, Insall RH: WASP Restricts Active Rac to Maintain Cells' Front-Rear Polarization. *Curr Biol* 2019, 29(24):4169-4182 e4164.

41. Bonfim-Melo A, Ferreira ER, Mortara RA: Rac1/WAVE2 and Cdc42/N-WASP Participation in Actin-Dependent Host Cell Invasion by Extracellular Amastigotes of Trypanosoma cruzi. *Front Microbiol* 2018, 9:360.

42. Tetley GJN, Szeto A, Fountain AJ, Mott HR, Owen D: Bond swapping from a charge cloud allows flexible coordination of upstream signals through WASP: Multiple regulatory roles for the WASP basic region. *J Biol Chem* 2018, 293(39):15136-15151.

43. Menotti M, Ambrogio C, Cheong TC, Pighi C, Mota I, Cassel SH, Compagno M, Wang Q, Dall'Olio R, Minero VG et al: Wiskott-Aldrich syndrome protein (WASP) is a tumor suppressor in T cell lymphoma. *Nat Med* 2019, 25(1):130-140.

44. Carabeo RA, Dooley CA, Grieshaber SS, Hackstadt T: Rac interacts with Abi-1 and WAVE2 to promote an Arp2/3-dependent actin recruitment during chlamydial invasion.
45. Egile C, Loisel TP, Laurent V, Li R, Pantaloni D, Sansonetti PJ, Carlier MF: Activation of the CDC42 effector N-WASP by the Shigella flexneri IcsA protein promotes actin nucleation by Arp2/3 complex and bacterial actin-based motility. *J Cell Biol* 1999, 146(6):1319-1332.

46. Espinoza-Sanchez S, Metskas LA, Chou SZ, Rhoades E, Pollard TD: Conformational changes in Arp2/3 complex induced by ATP, WASp-VCA, and actin filaments. *Proc Natl Acad Sci U S A* 2018, 115(37):E8642-E8651.

47. Hytti M, Piippo N, Korhonen E, Honkasoski P, Kaarniranta K, Kauppinen A: Fisetin and luteolin protect human retinal pigment epithelial cells from oxidative stress-induced cell death and regulate inflammation. *Sci Rep* 2015, 5:17645.

48. Sinha R, Srivastava S, Joshi A, Joshi UJ, Govil G: In-vitro anti-proliferative and anti-oxidant activity of galangin, fisetin and quercetin: role of localization and intermolecular interaction in model membrane. *Eur J Med Chem* 2014, 79:102-109.

49. Singh S, Singh AK, Garg G, Rizvi SI: Fisetin as a caloric restriction mimetic protects rat brain against aging induced oxidative stress, apoptosis and neurodegeneration. *Life Sci* 2018, 193:171-179.

50. Jacob S, Thangarajan S: Fisetin impedes developmental methylmercury neurotoxicity via downregulating apoptotic signalling pathway and upregulating Rho GTPase signalling pathway in hippocampus of F1 generation rats. *Int J Dev Neurosci* 2018, 69:88-96.

51. Wang M, Zhang W, Xu W, Shen Y, Du L: Optimization of genome shuffling for
high-yield production of the antitumor deacetylmycoepoxydiene in an endophytic fungus of mangrove plants. *Appl Microbiol Biotechnol* 2016, 100(17):7491-7498.

52. Xie W, Zhang W, Sun M, Lu C, Shen Y: Deacetylmycoepoxydiene is an agonist of Rac1, and simultaneously induces autophagy and apoptosis. *Appl Microbiol Biotechnol* 2018, 102(14):5965-5975.

53. Almatroodi SA, Alsahtli MA, Almatrodi A, Rahmani AH: Garlic and its Active Compounds: A Potential Candidate in The Prevention of Cancer by Modulating Various Cell Signalling Pathways. *Anticancer Agents Med Chem* 2019, 19(11):1314-1324.

54. Cobb-Abdullah A, Lyles LR, 2nd, Odewumi CO, Latinwo LM, Badisa VL, Abazinge M: Diallyl disulfide attenuation effect on transcriptome in rat liver cells against cadmium chloride toxicity. *Environ Toxicol* 2019, 34(8):950-957.

55. Su B, Su J, Zeng Y, Ding E, Liu F, Tan T, Xia H, Wu YH, Zeng X, Ling H et al: Diallyl disulfide inhibits TGFbeta1induced upregulation of Rac1 and betacatenin in epithelialmesenchymal transition and tumor growth of gastric cancer. *Oncol Rep* 2018, 39(6):2797-2806.

56. Xia L, Lin J, Su J, Oyang L, Wang H, Tan S, Tang Y, Chen X, Liu W, Luo X et al: Diallyl disulfide inhibits colon cancer metastasis by suppressing Rac1-mediated epithelial-mesenchymal transition. *Onco Targets Ther* 2019, 12:5713-5728.

57. Zhou Y, Su J, Shi L, Liao Q, Su Q: DADS downregulates the Rac1-ROCK1/PAK1-LIMK1-ADF/cofilin signaling pathway, inhibiting cell migration and invasion. *Oncol Rep* 2013, 29(2):605-612.
58. Yu MH, Yang TY, Ho HH, Huang HP, Chan KC, Wang CJ: Mulberry Polyphenol Extract Inhibits FAK/Src/PI3K Complex and Related Signaling To Regulate the Migration in A7r5 Cells. *J Agric Food Chem* 2018, 66(15):3860-3869.

59. Zhao X, Yang R, Bi Y, Bilal M, Kuang Z, Iqbal HMN, Luo Q: Effects of Dietary Supplementation with Mulberry (Morus alba L.) Leaf Polysaccharides on Immune Parameters of Weanling Pigs. *Animals (Basel)* 2019, 10(1).

60. Huang HP, Shih YW, Chang YC, Hung CN, Wang CJ: Chemoinhibitory effect of mulberry anthocyanins on melanoma metastasis involved in the Ras/PI3K pathway. *J Agric Food Chem* 2008, 56(19):9286-9293.

61. Akhtar N, Syed DN, Khan MI, Adhami VM, Mirza B, Mukhtar H: The pentacyclic triterpenoid, plectranthoic acid, a novel activator of AMPK induces apoptotic death in prostate cancer cells. *Oncotarget* 2016, 7(4):3819-3831.

62. Avila-Carrasco L, Majano P, Sanchez-Tomero JA, Selgas R, Lopez-Cabrera M, Aguilera A, Gonzalez Mateo G: Natural Plants Compounds as Modulators of Epithelial-to-Mesenchymal Transition. *Front Pharmacol* 2019, 10:715.

63. Bueno-Silva B, Alencar SM, Koo H, Ikegaki M, Silva GV, Napimoga MH, Rosalen PL: Anti-inflammatory and antimicrobial evaluation of neovestitol and vestitol isolated from Brazilian red propolis. *J Agric Food Chem* 2013, 61(19):4546-4550.

64. Hiep NT, Kwon J, Kim DW, Hwang BY, Lee HJ, Mar W, Lee D: Isoflavones with neuroprotective activities from fruits of Cudrania tricuspidata. *Phytochemistry* 2015, 111:141-148.

65. Gopal D, Muddebihalkar AG, Skariyachan S, C AU, Kaveramma P, Praveen U,
Shankar RR, Venkatesan T, Niranjan V: Mitogen activated protein kinase-1 and cell division control protein-42 are putative targets for the binding of novel natural lead molecules: a therapeutic intervention against Candida albicans. *J Biomol Struct Dyn* 2019:1-16.

66. Chen C, Wu W, Xu X, Zhang L, Liu Y, Wang K: Chain conformation and anti-tumor activity of derivatives of polysaccharide from Rhizoma Panacis Japonici. *Carbohydr Polym* 2014, **105**:308-316.

67. Huang Z, Zhang L: Chemical structures of water-soluble polysaccharides from Rhizoma Panacis Japonici. *Carbohydr Res* 2009, **344**(9):1136-1140.

68. Qi D, Yang X, Chen J, Li F, Shi X, Zhang C, Yang Z: Determination of chikusetsusaponin V and chikusetsusaponin IV in rat plasma by liquid chromatography-mass spectrometry and its application to a preliminary pharmacokinetic study. *Biomed Chromatogr* 2013, **27**(11):1568-1573.

69. Iijima R, Watanabe T, Ishiuchi K, Matsumoto T, Watanabe J, Makino T: Interactions between crude drug extracts used in Japanese traditional Kampo medicines and organic anion-transporting polypeptide 2B1. *J Ethnopharmacol* 2018, **214**:153-159.

70. Sasaki Y, Komatsu K, Nagumo S: Rapid detection of Panax ginseng by loop-mediated isothermal amplification and its application to authentication of Ginseng. *Biol Pharm Bull* 2008, **31**(9):1806-1808.

71. Chen X, Wu QS, Meng FC, Tang ZH, Chen X, Lin LG, Chen P, Qiang WA, Wang YT, Zhang QW *et al.* Chikusetsusaponin IVa methyl ester induces G1 cell cycle arrest, triggers apoptosis and inhibits migration and invasion in ovarian cancer cells.
72. Yuan K, Li X, Lu Q, Zhu Q, Jiang H, Wang T, Huang G, Xu A: Application and Mechanisms of Triptolide in the Treatment of Inflammatory Diseases-A Review. Front Pharmacol 2019, 10:1469.

73. Wang X, Zhao F, Lv ZM, Shi WQ, Zhang LY, Yan M: Triptolide disrupts the actin-based Sertoli-germ cells adherens junctions by inhibiting Rho GTPases expression. Toxicol Appl Pharmacol 2016, 310:32-40.

74. Chaotham C, Pongrakhananon V, Sritularak B, Chanvorachote P: A Bibenzyl from Dendrobium ellipsophyllum inhibits epithelial-to-mesenchymal transition and sensitizes lung cancer cells to anoikis. Anticancer Res 2014, 34(4):1931-1938.

75. Sritularak B, Duangrak N, Likhitwitayawuid K: A new bibenzyl from Dendrobium secundum. Z Naturforsch C J Biosci 2011, 66(5-6):205-208.

76. Chaotham C, Chanvorachote P: A bibenzyl from Dendrobium ellipsophyllum inhibits migration in lung cancer cells. J Nat Med 2015, 69(4):565-574.

77. Ku CY, Wang YR, Lin HY, Lu SC, Lin JY: Corosolic Acid Inhibits Hepatocellular Carcinoma Cell Migration by Targeting the VEGFR2/Src/FAK Pathway. PLoS One 2015, 10(5):e0126725.

78. Charoenrungruang S, Chanvorachote P, Sritularak B, Pongrakhananon V: Gigantol, a bibenzyl from Dendrobium draconis, inhibits the migratory behavior of non-small cell lung cancer cells. J Nat Prod 2014, 77(6):1359-1366.

Figure Legends
Figure 1. The schematic of osteoclastogenesis

The cytokines M-CSF and RANKL (from osteoblast) binds to its receptor cFms and RANKL present in osteoclast precursors, respectively. Then the M-CSF stimulates osteoclast precursors proliferation and inhibits their apoptosis. Besides that, RANKL interact with its receptor RANK in osteoclast precursor cells, then osteoclastogenesis is induced.

Figure 2. The schematic of molecular mechanisms of Rho GTPases Rac and Cdc42, and relevant therapeutic natural compounds.

During the osteoclastogenesis, after RANKL and RANK binding, the intracellular Rac1, Rac2 and Cdc42 are via GTP associate with podosomes regulation. However, these regulation effects might inhibited by various compounds (Left panel: inhibitory compounds for Rac1 and Rac2; Right panel: inhibitory compounds for Cdc42)
Table 1 The source, structure, cells or animal models and mechanisms of 10 natural compounds.
| Compound name | Source                      | Structure | Cell lines used for in vitro studies | Animal models | Dose    |
|---------------|----------------------------|-----------|-------------------------------------|---------------|---------|
| Fisetin       | *polyphe nolic molecul e of flavonoids* | Neuron    | Wistar Rats                         | 30mg/kg       |
| Deacetyl-mycoepoxydiene | *Phomo psis sp., of costal mangrove* | Human breast cancer MCF-7 cells | BABL/c mice | 5, 10, 20 mg/kg |
| Dially disulfide | garlic                      | Human Gastric cancer MGC803 cell line | BALB/c nude mice | 100mg/kg |
| Plectranthoic acid | *Ficus microcarpa* | Prostate cancer cell lines (DU145,PC3, NA22,NB26) | N/A            | N/A     |
| Cudraxanthone S | *Cudran ia cochinc hinensis* | N/A        | N/A                                 | N/A           |
| Panacis Japonici Rhizoma | *Panax japonicus C. A. Meyer Tritygium wilfordi i Hook. f.* | A2780 cell line | N/A                                 | N/A           |
| Triptolide    |                            | Sprague Dawley (SD) rats | Sprague Dawley (SD) rats | 100 mg/kg    |
| Compound          | Source/Description                                      | Target Cells                                    | Dose                  |
|-------------------|---------------------------------------------------------|-------------------------------------------------|-----------------------|
| TDB (4,5,40-trihydroxy-3,30-dimethoxybib enzy) | Dendrobium ellipsophyllum Tang and Wang               | Human lung cancer H292 cells                     | N/A                   |
| Corosolic acid    | Actinidia chinensis is, Thai orchid, Dendrobium draconis | Hepatocellular carcinoma cell lines (Huh7, HepG2 and Hep3B) | NOD/SCI D mice        | 5 mg/kg               |
| Gigantol          | Thai orchid, Dendrobium draconis                       | human lung carcinoma cells NCI-H460 and NCI-H292 | N/A                   | N/A                   |