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1. Introduction

Proper exercise regimes have been proposed for the postmenopausal women, the aged, astronauts and the patients after bed-rest in order to maintain bone mass and accelerate its recovery. The effects of mechanical loading on bone remodelling have become increasingly evident in recent years. In the process of mechanotransduction, the predominant osteocytes in bone tissue have the ability to sense mechanical strain and translate them into electrical or biochemical signals (Bonedwal and Johnson, 2008; Zhou et al., 2009), which can act on osteoblasts or osteoclasts contributing to bone turnover (Parfitt, 1984). Within seconds to minutes in response to mechanical stress, the signals are initiated from calcium channel activation and ATP, NO (nitric oxide) and PGE2 (prostaglandin E2) release, which is rapidly followed by the activation of the canonical Wnt/β-catenin pathway within 1 h (Bonedwal, 2006). Wnt/β-catenin pathway is required for bone maintenance and adaptation under mechanical loading (Galli et al., 2010).

The PI3K (phosphatidylinositol-3 kinase)/Akt [also known as PKB (protein kinase B)] signalling pathway is of interest because of both its recognized biological functions (Fresno Vara et al., 2004) and its cross-talk with Wnt/β-catenin pathway in bone cells (Xia et al., 2010). PI3K/Akt pathway can be activated by certain nuclear hormone (Schuur et al., 2001), biological factor (Harvey et al., 2004) and its cross-talk with Wnt/β-catenin pathway in bone cells not only on its own but also through the process of mechanotransduction of bone cells in a PI3K/Akt-dependent way. After describing downstream targets of FoxO, we speculate that FoxO would be involved in the positive effects of mechanical stimulation on bone cells directly through its target genes. We have also concisely represented the cross-talk between ROS (reactive oxygen species) and Wnt/β-catenin pathway, which leads us to hypothesize that the inhibition of FoxO caused by mechanical stress acts at the cross-roads between ROS and Wnt/β-catenin to regulate indirectly bone metabolism.

Keywords: bone cells; FoxO; mechanical stimulation; oxidative stress; PI3K/Akt; Wnt/β-catenin

Hypothesis

FoxO (forkhead box O) transcription factors, one of the main downstream mediators of PI3K (phosphatidylinositol-3 kinase)/Akt [also known as PKB (protein kinase B)] signal transduction pathway, play an important role in modulating cellular homeostasis. Recent studies have revealed the significance of FoxO in bone, the interaction of FoxO with β-catenin, along with mechanical stress-induced inactivation of FoxO via PI3K/Akt: We hypothesize that FoxO is a novel participant in mechanotransduction of bone cells in a PI3K/Akt-dependent way. After describing downstream targets of FoxO, we speculate that FoxO would be involved in the positive effects of mechanical stimulation on bone cells directly through its target genes. We have also concisely represented the cross-talk between ROS (reactive oxygen species) and Wnt/β-catenin pathway, which leads us to hypothesize that the inhibition of FoxO caused by mechanical stress acts at the cross-roads between ROS and Wnt/β-catenin to regulate indirectly bone metabolism.
regulator of FoxO-mediated transcription (Behrens et al., 1996). Activated Akt directly phosphorylates FoxO (1, -3a and -4) at three known sites (Thr32, Ser253 and Ser315) that are conserved from Caenorhabditis elegans to mammals, which promotes the translocation of FoxO to the cytosol, thereby repressing its transcriptional activity (Van Der Heide et al., 2004). FoxOs are mechanosensitive and their transcriptional activity is regulated by mechanical stimuli via PI3K/Akt, and subsequently the phosphorylation of Akt (Danciu et al., 2003, 2004; Pardo et al., 2008). In osteoblasts and osteocytes, not only mechanical stimulation but also the PGE2 release and Ca2+ flux can induce the recruitment and activation of PI3K, and subsequently the phosphorylation of Akt (Danciu et al., 2003; Bhattacharjee et al., 2009). Particularly in mouse pre-osteoblastic cells (MC3T3-E1), FoxO is phosphorylated and inactivated by cyclic stretch via PI3K/Akt pathway (Danciu et al., 2003). However, there are no reports about the subsequent biological effects of inactivated FoxO caused by mechanical stress/PI3K/Akt on bone cells.

2. Hypothesis

We hypothesize that FoxO is a novel participant in mechanotransduction of bone cells in a PI3K/Akt-dependent way. Specifically, we propose that mechanical stress-mediated PI3K/Akt/FoxO signal pathway is directly associated with the proliferation and apoptosis of bone cells, and functions at the cross-roads between oxidative stress and Wnt/β-catenin pathway to indirectly regulate bone homeoeostasis.

2.1. FoxO: a potential participant in mechanical stimulation-induced proliferation and anti-apoptosis of bone cells?

Activation of FoxO transcription factors is associated with cell-cycle arrest and apoptosis (Greer and Brunet, 2005), which implies that the suppression of FoxO may induce proliferation and decrease apoptosis. To the best of our knowledge, proper mechanical stimulus contributes to the activity and proliferation of bone cells and stress-induced PI3K/Akt/FoxO has been identified in osteoblasts (Danciu et al., 2003). FoxO may serve as a potential participant in the anabolic effects of mechanical stress on bone cells via the downstream targets.

The cyclin kinase inhibitor p27Kip1, a prominent FoxO target, acts as a potent modulator of cell cycle progression in osteoblasts (Drissi et al., 1999). COX-2, up-regulated by mechanical strain or PGE2 treatment, facilitates osteoblasts proliferation via the inhibition of p27Kip1 through PTEN (phosphatase and tensin homologue deleted on chromosome 10)/PI3K/Akt/FoxO signal pathway (Li et al., 2011). Anti-inflammatory drugs also suppress the proliferation of human osteoblasts partly through inactivating Akt, activating FoxO3a, and eventually up-regulating p27Kip1 (Li et al., 2010). Therefore mechanical stress-induced proliferation of bone cells is probably associated with the activation of PI3K/Akt, inactivation of FoxO and inhibition of p27Kip1.

Mechanical strain delivers anti-apoptotic signals to human gingival fibroblasts via FasL through PI3K/Akt/FoxO pathway (Danciu et al., 2004). FoxO1 and FoxO3a as pro-apoptotic factors were identified in osteoblasts. The underlying mechanism was further assessed by knockdown of FoxO1 with RNA interference, which significantly inhibited pro-apoptotic genes [TNFα (tumour necrosis factor α), FADD (Fas-associated death domain) and caspase 3, 8, and 9] and reduced apoptosis of MC3T3 cells caused by cytokines (Behl et al., 2008). In addition, the Akt/FoxO3a/Bim-axis has a novel role in osteoblast apoptosis. This finding shows that Akt phosphorylated FoxO3a to inhibit its nuclear localization, resulting in impaired transactivation of Bim which is also a potent pro-apoptotic molecule in osteoblasts (Kawamura et al., 2007). Thus inactivated FoxO1 and FoxO3a may be involved in the anti-apoptotic effects of mechanical stress on osteoblasts via modulating these candidate apoptotic molecules.

2.2. FoxO: a promising candidate to serve as molecular links among oxidative stress, mechanical stimulation and Wnt/β-catenin?

The increase of oxidative stress with aging or sex hormone deficiency is associated with bone loss. ROS (reactive oxygen species) can disrupt 14-3-3 binding to FoxO via JNK (c-Jun N-terminal kinase), enable FoxO’s entrance into nucleus, and induce its transcriptional activation (Figure 1) (Nakae et al., 2008; Morrison, 2009). FoxO activation leads to up-regulation of Mn-SOD, catalase and Gadd45 and eventually antagonizes the anabolic effects of Wnt/β-catenin on bone through the diversion of β-catenin from TCF (T-cell factor)/LEF (lymphoid enhancer factor) to FoxO-mediated transcription (Manolagas and Almeida, 2007). On the contrary, mechanical stimulation can activate the Wnt/β-catenin pathway and induce bone mass production (Robinson et al., 2006). As previously stated, mechanical stress can phosphorylate FoxO via PI3K/Akt, resulting in its nuclear exclusion. It implies that mechanical strain-activated Akt and ROS-activated JNK have opposing effects on FoxO subcellular localization. Therefore mechanical stress is most likely to increase β-catenin/TCF interaction and lower FoxO interaction with β-catenin through regulating FoxO shuttling from the nucleus to the cytoplasm. It eventually results in converting the adverse effects of ROS to the beneficial effects of Wnt on bone.

Phosphorylation of FoxO through mechanical stress facilitates FoxO nuclear export and 14-3-3 may be involved in this process via binding to FoxO (Figure 1). The highly conserved 14-3-3 protein family (β, γ, ε, ζ, σ, τ and η) is important for intracellular signal transduction events in all eukaryotic cells (Brunet et al., 2002). The binding of 14-3-3 to phosphorylated FoxO contributes to the nuclear export of FoxO, which retains them in the cytoplasm (Nakae et al., 2008). Mechanical stress may, to some extent, recover this binding via PI3K/Akt/FoxO to inhibit FoxO nuclear import. Therefore localization of FoxOs in the cytoplasm suppresses FoxO-mediated transcription induced by ROS and leads to β-catenin shifting to TCF/LEF. Together, mechanical stimulation may have the potential to adjust the balance between ROS and Wnt/β-catenin via regulating the localization, and transcription of FoxOs. FoxOs are expected to be pivotal molecular links among ROS, mechanical stimulation and Wnt/β-catenin in bone cells.

Previous studies have suggested that the limited pool of β-catenin is susceptible to make the diverting β-catenin from TCF/LEF to FoxO...
under oxidative stress (Hoogeboom and Burgering, 2009). This suggests that increasing β-catenin will alleviate the adverse effects of ROS on Wnt. Mechanical loading can inhibit GSK-3β via PI3K/Akt to increase β-catenin accumulation in bone cells (Figure 1) (Santos et al., 2010; Xia et al., 2010). We think that PI3K/Akt/GSK-3β pathway will also become a switch access to the balance between ROS and Wnt by increasing β-catenin levels.

3. Conclusion

Owing to growing evidence that FoxO is a novel and pivotal molecule in bone metabolism, it is worth investigating the contribution of FoxO to bone cells and the underlying mechanisms. Mechanical stimulation is a critical player in bone remodelling, and the mechanical stress-induced signalling pathway can control FoxO activity. This hypothesis, therefore, directed us to focus on the possible actions of FoxO on bone cells under mechanical loading. Specifically, mechanical stress-mediated PI3K/Akt/FoxO signal pathway is proposed to exert its influences directly by targeting genes of FoxO, as well as indirectly by cross-talk with ROS and Wnt/β-catenin. Therefore FoxO is expected to be a valuable target of therapeutic treatment for bone diseases.

Author contribution

Yuanyuan Ma contributed to the conception of the hypothesis and wrote the manuscript. Hang Wang gave administrative, technical and financial support. Both authors were responsible for the revision of the paper and approved the final version.

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