Involvement of Androgen-Mediated Osteopontin in Bone Remodeling via Regulation of Osteoclast-Specific Actin Ring

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Purpose: Osteoporosis and secondary fracture in men cause significant morbidity and mortality, due to the global trends of increased longevity. Although the direct role of androgens independent of estrogen in bone growth was demonstrated by mouse models, the underlying molecular mechanism in periosteal bone formation and remodeling remains elusive. In the present study, we performed a quantitative proteomics analysis to comprehensively investigate the differential expressed proteins during osteoclast and osteoblast differentiation in the presence of 17β-estradiol (β-E2) or testosterone. In addition, we emphasize the pivotal role of osteopontin (OPN) in androgen-mediated bone remodeling.

Methods: The osteoclast and osteoblast differentiation were induced standard differentiation medium. The mature cells were digested to proteins and labeled by tanden mass tags. After the high performance liquid chromatography separation, the labeled samples were dissolved for subsequent liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis. Proteins were identified using Proteome Discoverer 2.1 software with the SEQUEST search engine. Gene ontology (GO) analysis, pathway mapping, and protein networks were performed. The MS results for the identified proteins were validated by Western blotting and representative mass spectral analysis. The relation between testosterone and OPN in human serum was measured by enzyme-linked immunosorbent assay (ELISA), and the effects of OPN on regulation of actin ring were identified by in vitro studies.

Results: Bioinformatics analysis and in vitro studies confirmed that testosterone additionally attenuated osteoclast differentiation with the upregulation of STAT1-associated immune responses. In contrast, testosterone strongly promoted osteoblast formation with the enhancement of focal adhesion and indirectly suppressed osteoclast differentiation in the later stages of osteogenesis. Additionally, the OPN expression was continued repressed in mature osteoblast by testosterone, which is a key factor for regulating the osteoclast precursors to accelerate their differentiation to actin ring-enriched bone-resorbing cells.

Conclusion: This information will be helpful in elucidating the functions of androgen in mediation of bone remodeling as well as providing a basis for the development of strategies for osteoporosis prevention and therapy in men. Despite the rapid advances in our knowledge regarding the regulation of bone remodeling, the specific roles of androgen on cells of the osteoclast and osteoblast lineage await future studies.