ANTI-APOPTOTIC EFFECT OF PTH (1-34) VIA PI3K-AKT-BAD SIGNALING PATHWAY IN OSTEOBLAST-LIKE MG63 CELLS

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ABSTRACT INTRODUCTION:
Parathyroid hormone (PTH) binds to its receptor, parathyroid hormone 1 receptor (PTH1R) in osteoblastic cells to regulate bone remodeling and calcium homeostasis. While prolonged exposure to PTH causes increased bone resorption, intermittent injections of PTH have an anabolic effect on bone. The molecular mechanisms regulating these processes are still largely unknown. It is widely believed that the anabolic effect of PTH is the result of increased osteoblast differentiation and decreased osteoblast apoptosis (1).
On the other hand, it is well known that the activation of phosphatidylinositol 3'-kinase (PI3K)-Akt-Bad signaling pathway induces anti-apoptotic effect in several cell lines (2).
Therefore, we investigated whether anti-apoptotic effect of PTH (1-34) was mediated via PI3K-Akt-Bad signaling pathway in osteoblast-like MG63 cells.
METHODS:
Immunoblot analysis:
MG63 cells were seeded in 100-mm dishes at 1.2×10^4 cells/cm^2 in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and incubated for a few days until cells reached 80% confluency. The cells were then switched to serum free DMEM and incubated for 16 hours. Some cells were harvested at 5, 10, 15, 20, and 30 minutes after treatment with or without PTH (1-34), others were harvested at 20 minutes after stimulated by PI3K inhibitor (wortmannin or LY294002) or actinomycin-D with or without 10^{-7} M PTH (1-34). Proteins derived from cell lysates were loaded into 10% SDS-PAGE gel, resolved by electrophoresis, and transferred to a hydrophobic polyvinylidene difluoride membrane. Membrane was blocked in TTBS buffer (100 mM Tris-HCl pH 7.5, 150 mM NaCl, and 0.1% Tween-20) with 3% skim milk, and then incubated with primary antibodies (anti-phospho-Akt antibody or ant-Akt antibody or anti-Bad antibody) followed by enhanced chemiluminescence detection using horseradish peroxidase-conjugated second antibodies.
Immunoprecipitation experiments:
Proteins derived from cell lysates were clarified and incubated with anti-PTH1R antibody on a rocking platform at 4 ℃ overnight. The immune complexes were collected with Protein G beads for 1 hour at 4 ℃. Immunoprecipitated proteins were then analyzed by SDS-PAGE and subjected to Western blot analysis employing anti-PI3K (p85 subunit) antibody.
Determination of osteoblast apoptosis:
MG63 cells were seeded in 12 well dishes at 5.0×10^3 cells/cm^2 in DMEM supplemented with 10% FBS and incubated for a few days until cells reached 80% confluency. The cells were switched to serum free DMEM and stimulated by PI3K inhibitor (wortmannin or LY294002) or actinomycin-D with or without 10^{-7} M PTH (1-34) for 48 hours. Apoptotic cells were detected by the terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) staining.
RESULTS SECTION:
Akt was phosphorylated by PTH (1-34) stimulation within 5 minutes. This phosphorylation of Akt was blocked by PI3K inhibitors, Wortmannin and LY294002, but not by PKA inhibitor, H89 (Fig.1). Bad was also phosphorylated by PTH (1-34) stimulation.
When cell lysates were immunoprecipitated with anti-PTH1R, followed by immunoblotting with anti-PI3K (p85 subunit), PI3K (p85 subunit) could be detected only after PTH (1-34) stimulation (Fig.2).
The proportion of osteoblasts undergoing apoptosis, as determined by TUNEL staining, was decreased in cells receiving PTH (1-34). The average proportion of apoptotic cells was 4% after PTH (1-34) stimulation, as compared with 16% after vehicle stimulation. This anti-apoptotic effect of PTH (1-34) was attenuated by PI3K inhibitors, Wortmannin and LY294002 (Fig.3).
DISCUSSION:
The molecular effects of PTH are mediated by the G-protein-dependent, membrane receptor, PTH1R, found in osteoblasts and renal tubular cells. It is well known that the binding of the ligand to the PTH1R activates adenylate cyclase and a number of phospholipases (3).
Several lines of evidence support the notion that the anti-apoptotic effect of PTH on osteoblasts is mediated by cAMP. But there have been no papers that reported the effects of PTH on PI3K-Akt-Bad signaling pathway in osteoblasts (4).
In this study, we first demonstrated that PTH directly activates PI3K via PTH1R and mediates the anti-apoptotic effect. PI3K-Akt-Bad signaling pathway may be involved in anti-apoptotic effect of PTH in osteoblast-like MG63 cells and nominated for the therapeutic strategy, targeting bone degenerated disease, including osteoporosis.

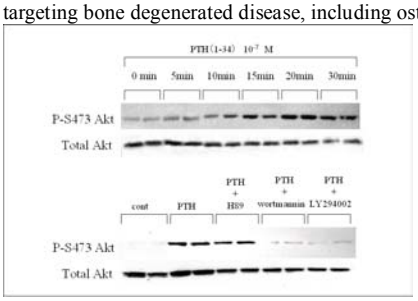

Fig. 1. Phosphorylation of Akt by PTH stimulation

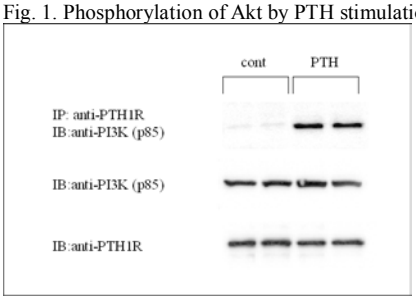

Fig. 2. PTH1R directly binds to PI3K by PTH stimulation

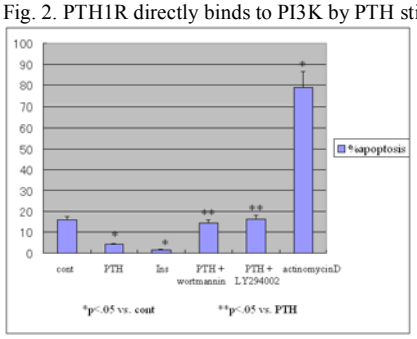

Fig. 3. Detection of apoptotic cells by TUNEL staining

REFERENCES:

1. Dempster, D.W., Cosman, F., Parisien, M., and Shen, V. 1993. Anabolic actions of parathyroid hormone on bone. Endocr Rev. 14:690-709.
2. Craddock, B.L., Orchiston, E.A., Hinton, H.J., Welham, M.J. 1999. Dissociation of apoptosis from proliferation, protein kinase B activation, and BAD phosphorylation in interleukin-3-mediated phosphoinositide 3-kinase signaling. J Biol Chem. 274:10633-40.
3. Turner, P.R., Bencsik, M., Malecz, N., Christakos, S., and Nissenson, R.A. 1998. Apoptosis mediated by the PTH/PTHrP receptor: role of JNK and calcium signaling pathways. Bone. 23: S155.
4. Machwate, M., Rodan, S.B., Rodan, G.A., and Harada, S.I. 1998. Sphingosine kinase mediates cAMP suppression of apoptosis in rat periosteal cells. Mol Pharmacol. 54:70-77