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研究課題名 (和文) 副甲状腺細胞内における副甲状腺ホルモン断片化調節機構の研究

研究課題名 (英文) Truncation of parathyroid hormone in parathyroid cells

研究代表者

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研究成果の概要 (和文)：副甲状腺細胞は細胞膜上のカルシウム感知受容体 (CaR) により細胞外カルシウム濃度に応じて副甲状腺ホルモン (PTH) の分泌量を調節し、PTH の N 端断片化はその一つの機構である。副甲状腺細胞に細胞内カルシウムキレーターを添加すると PTH 分泌抑制は解除されたが、N 端断片化には変化を認めなかった。PTH 分子 N 端断片化機構は PTH 分泌抑制機構における細胞内シグナル伝達系とは異なることが示唆された。

研究成果の概要 (英文)：Extracellular calcium concentration regulates parathyroid hormone (PTH) secretion via calcium-sensing receptor (CaR) on the parathyroid cell surface. N-terminal truncation of PTH is also regulated by extracellular calcium concentration. The N-terminal truncation is thought to be one of the regulatory mechanisms to secrete 1-84PTH from parathyroid cells, however, signal pathways of the mechanism in the cells are not yet reported. We attempt to determine whether the pathways of N-terminal truncation are identical from those of (1-84)PTH secretion in primary cultured human parathyroid cells. It is suggested that the N-terminal truncation of PTH regulated by extracellular calcium concentration has at least partly independent intracellular signaling systems from the (1-84)PTH secretion in human parathyroid cells.

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1. 研究開始当初の背景

PTHは、細胞外カルシウム濃度により厳密にその分泌が調節されており、血清カルシウム濃度の調節維持に重要な役割を演じている。細胞外カルシウムによるPTHの分泌調節機構は、PTH遺伝子の発現、PTH mRNAの安定性、副甲状腺細胞内でのPTH分子の断片化、副甲状腺細胞増殖といった多くの段階で調節されている。従来より副甲状腺細胞内でのPTH分子の断片化は、N端より33、34番、および36、37番といったPTH分子中央部(mid region)における分解機構について検討が行われてきた。

我々は、臨床研究において維持透析患者におけるN端断片化PTHの存在様式について検討を行い、PTH(1-84)とN端断片化PTHの比率が血清カルシウム濃度と負に相関することを報告した(Inaba M et al. Significance of Bio-intact PTH(1-84) assay in hemodialysis patients. *Osteoporos Int* 16(5):517-25, 2005)。この論文は、PTHのN端断片化現象が血清カルシウム濃度依存性であることを最初に報告したものである。

これまで我々は、副甲状腺からPTHが分泌されるに至る様々な段階で、CaRを刺激することによりPTHの分泌を抑制できることを、原発性副甲状腺機能亢進症モデルマウス(PCマウス)や初代培養副甲状腺細胞を用いて示してきた。特にシナカルセトによるCaRの刺激で、PTH分泌が抑制されることを下記の検討で示してきた。

PCマウスを用いた検討(Kawata T, et al. Relationship between parathyroid calcium-sensing receptor expression and potency of the calcimimetic, cinacalcet, in suppressing parathyroid hormone secretion in an in vivo murine model of primary hyperparathyroidism. *Eur J Endocrinol* 153(4):587-594, 2005)、初代培養副甲状腺細胞を用いた検討(Kawata T, Imanishi Y, et al. Direct in vitro evidence of the suppressive effect of cinacalcet HCl on parathyroid hormone secretion in human parathyroid cells with pathologically reduced calcium-sensing receptor levels. *J Bone Miner Metab* 24(4):300-6, 2006)

2. 研究の目的

副甲状腺細胞内において生じる副甲状腺ホルモン(PTH)の断片化は、既に合成したPTH分子を分泌直前に分解するということより、副甲状腺細胞に対する早期のPTH分泌抑制刺激に反応する機構であると考えられる。PTHのN端断片化機構の少ないとも一部は、副甲状腺細胞膜上に存在するカルシウム感受

容体(CaR)を介していることを明らかになっている。PTHのN端断片化反応は、PTH分泌抑制機構と同様にCaRを介して調節されると考えられるが、その細胞内メカニズムについての報告は皆無で、PTH分泌抑制機構との関連についての報告もない。今回、PTHのN端断片化機構が、既知のPTH分泌調節機構と共通の制御をうけているのか、それともPTH分泌調節機構とは独立した独自の機構を有しているのかについて、ヒト副甲状腺の初代培養系を用いて検討した。

3. 研究の方法

本研究では、二次性副甲状腺機能亢進症を対象とし、日本透析医学会のガイドラインに準じて副甲状腺摘出術に至った症例における副甲状腺組織の初代培養系を用いて実験を行った。本研究は大阪市立大学大学院医学研究科の倫理委員会で承認を受けており、すべての対象者には本研究の趣旨を十分に説明され、理解を得た上で、文書による同意が得られている。

4. 研究成果

副甲状腺細胞は細胞膜上のカルシウム感知受容体(calcium sensing-receptor; CaR)により細胞外カルシウム濃度に応じて副甲状腺ホルモン(parathyroid hormone; PTH)の分泌量を調節している。近年、細胞外カルシウム濃度の上昇とともにN端側の数ペプチドが欠損したPTH断片の分泌割合が増加することが示された。このようなN端断片化PTHは生理活性を有さないことから、PTH分子のN端断片化反応はPTH分泌の調節機構の一つであると考えられる。今回、PTH分子のN端断片化機構について、既知の分泌抑制機構と共通の制御を受けているのか否かに注目し、ヒト副甲状腺組織初代培養系を用いて検討を行った。

副甲状腺摘出術(PTX)に至った二次性副甲状腺機能亢進症の副甲状腺組織初代培養系を用い、細胞内カルシウムキレーター(BAPTA-AM)の添加による、細胞外カルシウム濃度依存性のPTH分子のN端断片化反応への影響を検討した。BAPTA-AM添加にて細胞外高カルシウム環境下でのPTH分泌抑制は解除されたが、分泌されたN端断片化PTHの割合には変化を認めなかった。PTH分子N端断片化機構の細胞内メカニズムはPTH分泌抑制機構における細胞内シグナル伝達系とは異なることが示唆された。

5. 主な発表論文等

(研究代表者、研究分担者及び連携研究者には下線)

[雑誌論文] (計 57 件)

- ① Kurajoh M 総 10 名, Inaba M(2 番目), Imanishi Y(6 番目): Reduction of whole PTH/intact PTH ratio as a predictor of bone metabolism in cinacalcet treatment of hemodialysis patients with secondary hyperparathyroidism, *Osteoporos Int* 2011, 22:923-930, 査読有
- ② Kawano N 総 12 名, Inaba M(12 番目): Association of serum TRAIL levels with atherosclerosis in patients with type 2 diabetes mellitus, *Diabetes research and clinical practice* 2011 91:316-320, 査読有
- ③ Kawaguchi Y, 総 7 名, Inaba M(3 番目): Different impacts of neck circumference and visceral obesity on the severity of obstructive sleep apnea syndrome, *Obesity* 2011 19:276-282, 査読有
- ④ Ueno H, 総 12 名, Inaba M(8 番目): Advanced glycation end products, carotid atherosclerosis, and circulating endothelial progenitor cells in patients with end-stage renal disease, *Metabolism* 2011 60:453-459, 査読有
- ⑤ Tamori A, 総 11 名, Inaba M(8 番目): Prospective study of reactivation of hepatitis B virus in patients with rheumatoid arthritis who received immunosuppressive therapy: evaluation of both HBsAg-positive and HBsAg-negative cohorts. *Journal of gastroenterology* 2011, 46:556-564, 査読有
- ⑥ Yajima A, Inaba M, 他 5 名: Increased osteocyte death and mineralization inside bone after parathyroidectomy in patients with secondary hyperparathyroidism, *J Bone Miner Res* 2010, 25:2374-2381, 査読有
- ⑦ Ishii A 総 8 名, Imanishi Y(2 番目), Inaba M(7 番目): The administration of an active vitamin D₃ analogue reduced the serum concentrations of 1-84 and truncated parathyroid hormone in pseudohypoparathyroidism type Ib patients, *Endocr J* 2010, 57:609-614, 査読有
- ⑧ Ishii A 総 19 名, Imanishi Y(2 番目), Inaba M(18 番目): The levels of somatostatin receptors in causative tumors of oncogenic osteomalacia are insufficient for their agonist to normalize serum phosphate levels, *Calcif Tissue Int* 2010, 86:455-462, 査読有
- ⑨ Inaba M, 総 9 名, Imanishi Y(4 番目): Poor muscle quality rather than reduced lean body mass is responsible for the lower serum creatinine level in hemodialysis patients with diabetes mellitus, *Clin Nephrol* 2010, 74:266-272, 査読有
- ⑩ Ichii M 総 11 名, Inaba M(10 番目): Decreases in parathyroid gland volume after cinacalcet treatment in hemodialysis patients with secondary hyperparathyroidism, *Nephron Clin Pract* 2010, 115:c195-202, 査読有
- ⑪ Emoto M 総 11 名, Inaba M(10 番目): Fetuin-A and atherosclerotic calcified plaque in patients with type 2 diabetes mellitus, *Metabolism* 2010, 59:873-878, 査読有
- ⑫ Ban A, Inaba M, 他 5 名: Time-course of health status in patients with rheumatoid arthritis during the first year of treatment with infliximab, *Biomed Pharmacother* 2010, 64:107-112, 査読有
- ⑬ Yamada S, 総 8 名, Inaba M (2 番目), Imanishi Y(4 番目): Association of glycated albumin, but not glycated hemoglobin, with calcaneus quantitative ultrasound in male hemodialysis patients with type 2 diabetes mellitus, *Metabolism* 2010, 59:390-394, 査読有
- ⑭ Ueno H, 総 14 名, Inaba M(13 番目): Receptor for advanced glycation end-products (RAGE) regulation of adiposity and adiponectin is associated with atherogenesis in apoE-deficient mouse, *Atherosclerosis* 2010, 211:431-436, 査読有
- ⑮ Ueno H, 総 12 名, Inaba M(11 番目): Regions of arterial stenosis and clinical factors determining transcutaneous oxygen tension in patients with peripheral arterial disease, *J Atheroscler Thromb* 2010, 17:858-869, 査読有
- ⑯ Tsuchikura S, 総 9 名, Inaba M(8 番目): Serum C-reactive protein and thioredoxin levels in subjects with mildly reduced glomerular filtration rate, *BMC Nephrol* 2010, 11:7, 査読有
- ⑰ Tamori A, 総 10 名, Imanishi Y (9 番目): Add-on combination therapy with

- adefovir dipivoxil induces renal impairment in patients with lamivudine-refractory hepatitis B virus, *J Viral Hepat* 2010, 17:123-129, 査読有
- ⑱ Takada J, Miki T, Imanishi Y, 他 7 名: Effects of raloxifene treatment on the structural geometry of the proximal femur in Japanese women with osteoporosis, *J Bone Miner Metab* 2010, 28:561-567, 査読有
- ⑲ Sugiguchi S, Goto H, Inaba M, Nishizawa Y: Preferential reduction of bone mineral density at the femur reflects impairment of physical activity in patients with low-activity rheumatoid arthritis, *Mod Rheumatol* 2010, 20:69-73, 査読有
- ⑳ Shoji T, 総 8 名, Inaba M(7 番目): Arterial stiffness predicts cardiovascular death independent of arterial thickness in a cohort of hemodialysis patients, *Atherosclerosis* 2010, 210:145-149, 査読有
- ㉑ Shima H, 総 13 名, Inaba M(10 番目): Cerebral microbleeds in predialysis patients with chronic kidney disease, *Nephrol Dial Transplant* 2010, 25:1554-1559, 査読有
- ㉒ Nagasaki T, Inaba M, 他 8 名: Thyroid blood flow as a useful predictor of relapse of Graves' disease after normal delivery in patients with Graves' disease, *Biomed Pharmacother* 2010, 64:113-117, 査読有
- ㉓ Mori K, 総 11 名, Inaba M(10 番目): Effects of pravastatin on serum osteoprotegerin levels in patients with hypercholesterolemia and type 2 diabetes, *Angiology* 2010, 61:86-91, 査読有
- ㉔ Mori K, 総 9 名, Inaba M(7 番目): Association of serum TRAIL level with coronary artery disease, *Thromb Res* 2010, 125:322-325, 査読有
- ㉕ Mori K, 総 11 名, Inaba M(9 番目): Fetuin-A is associated with calcified coronary artery disease, *Coron Artery Dis* 2010, 21:281-285, 査読有
- ㉖ Koyama H, 総 15 名, Inaba M(4 番目): Fatigue is a predictor for cardiovascular outcomes in patients undergoing hemodialysis, *Clin J Am Soc Nephrol* 2010, 5:659-666, 査読有
- ㉗ Kobayashi I, 総 9 名, Inaba M(8 番目): Geriatric Nutritional Risk Index, a simplified nutritional screening index, is a significant predictor of mortality in chronic dialysis patients, *Nephrol Dial Transplant* 2010, 25:3361-3365, 査読有
- ㉘ Ueno H 総 12 名, Inaba M(8 番目): Advanced glycation end products, carotid atherosclerosis, and circulating endothelial progenitor cells in patients with end-stage renal disease, *Metabolism* 2010, 60:453-459
- ㉙ Okamoto K 総 8 名, Inaba M(2 番目), Imanishi Y(7 番目): Beneficial effect of risedronate on arterial thickening and stiffening with a reciprocal relationship to its effect on bone mass in female osteoporosis patients: a longitudinal study, *Life sciences* 2010, 87:686-691
- ㉚ Mima Y 総 11 名, Inaba M(11 番目): Different effects of diabetic autonomic neuropathy on regional transcutaneous oxygen tension in patients with peripheral arterial disease. *Osaka city medical journal* 2010, 56:27-36
- ㉛ Tsujimoto Y, 総 11 名, Inaba M(8 番目): Poor glycemic control is a significant predictor of cardiovascular events in chronic hemodialysis patients with diabetes, *Ther Apher Dial* 2009, 13:358-365, 査読有
- ㉜ Onoda N, 総 10 名, Inaba M(5 番目): Cushing's syndrome by left adrenocortical adenoma synchronously associated with primary aldosteronism by right adrenocortical adenoma: report of a case, *Endocr J* 2009, 56:495-502, 査読有
- ㉝ Okuno S, 総 8 名, Inaba M(7 番目) Serum β_2 -microglobulin level is a significant predictor of mortality in maintenance haemodialysis patients, *Nephrol Dial Transplant* 2009, 24:571-577, 査読有
- ㉞ Ohata Y, 総 9 名, Imanishi Y (6 番目): Severe arterial hypertension: a possible complication of McCune-Albright syndrome, *Eur J Pediatr* 2009, 168:871-876, 査読有
- ㉟ Nagayama H, Inaba M, 他 5 名: Glycated albumin as an improved indicator of glycemic control in hemodialysis patients with type 2 diabetes based on fasting plasma glucose and oral glucose tolerance test, *Biomed Pharmacother* 2009, 63:236-240, 査読有
- ㊱ Nagasaki T, Inaba M, 他 8 名: Decrease of brachial-ankle pulse wave velocity in

- female subclinical hypothyroid patients during normalization of thyroid function: a double-blind, placebo-controlled study, *Eur J Endocrinol* 2009, 160:409-415, 査読有
- ③⑦ Maeno Y, Inaba M, 他 6 名 Significant association of fracture of the lumbar spine with mortality in female hemodialysis patients: a prospective observational study, *Calcif Tissue Int* 2009, 85:310-316, 査読有
- ③⑧ Lee E, 総 13 名, Inaba M(12 番目): The combination of IMT and stiffness parameter beta is highly associated with concurrent coronary artery disease in type 2 diabetes, *J Atheroscler Thromb* 2009, 16:33-39, 査読有
- ③⑨ Kohno K, Inaba M, 他 6 名: Association of reduction in bone mineral density with mortality in male hemodialysis patients, *Calcif Tissue Int* 2009, 84:180-185, 査読有
- ④⑩ Kobayashi I, 総 11 名, Inaba M(9 番目): Renal biopsy in a patient with haemophilia A and cryoglobulinaemic membranoproliferative glomerulonephritis associated with hepatitis C virus infection, *NDT Plus* 2009, 2:373-375, 査読有
- ④⑪ Kanazawa I, 総 12 名, Imanishi Y(4 番目), Inaba M(9 番目): Osteosarcoma in a pregnant patient with McCune-Albright syndrome, *Bone* 2009, 45:603-608, 査読有
- ④⑫ Ishimura E, 総 12 名, Inaba M(11 番目): Glycemic control and survival of diabetic hemodialysis patients--importance of lower hemoglobin A1C levels, *Diabetes Res Clin Pract* 2009, 83:320-326, 査読有
- ④⑬ Imanishi Y, Inaba M, 他 2 名: Cinacalcet in hyperfunctioning parathyroid diseases, *Ther Apher Dial* 2009, 13 Suppl 1:S7-S11, 査読有
- ④⑭ Imanishi Y, Inaba M, 他 2 名: Animal models of hyperfunctioning parathyroid diseases for drug development, *Expert Opin Drug Discov* 2009, 4:727-740, 査読有
- ④⑮ Bando H, 総 9 名, Imanishi Y(7 番目): Severe hypophosphatemic osteomalacia with fanconi syndrome, renal tubular acidosis, vitamin D deficiency and primary biliary cirrhosis, *Intern Med* 2009, 48:353-358, 査読有
- ④⑯ Yamada S, Inaba M, 他 5 名: Association of glycated albumin, but not glycated hemoglobin, with peripheral vascular calcification in hemodialysis patients with type 2 diabetes, *Life Sci* 2008, 83:516-519, 査読有
- ④⑰ Yamada S, 総 7 名, Inaba M(2 番目), Imanishi Y(5 番目): Utility of serum tartrate-resistant acid phosphatase (TRACP5b) as a bone resorption marker in patients with chronic kidney disease: independence from renal dysfunction, *Clin Endocrinol (Oxf)* 2008, 69:189-196, 査読有
- ④⑱ Yajima A, Inaba M, 他 2 名: Bone formation by remodeling is more active than remodeling after parathyroidectomy, *Kidney Int* 2008, 74:775-781, 査読有
- ④⑲ Shidara K, Inaba M, 他 7 名: Serum Levels of TRAP5b, a New Bone Resorption Marker Unaffected by Renal Dysfunction, as a Useful Marker of Cortical Bone Loss in Hemodialysis Patients, *Calcif Tissue Int* 2008, 82:278-287, 査読有
- ⑤⑰ Riches PL, Imanishi Y, 他 2 名: Clinical and Biochemical Response of TNFRSF11A-Mediated Early-Onset Familial Paget Disease to Bisphosphonate Therapy, *Calcif Tissue Int* 2008, 83:272-275, 査読有
- ⑤⑱ Nishizawa Y, Inaba M, 他 8 名: Reference intervals of serum tartrate-resistant acid phosphatase type 5b activity measured with a novel assay in Japanese subjects, *J Bone Miner Metab* 2008, 26:265-270, 査読有
- ⑤⑲ Mori K, 総 10 名, Inaba M(9 番目): Effects of pioglitazone on serum fetuin-A levels in patients with type 2 diabetes mellitus, *Metabolism* 2008, 57:1248-1252, 査読有
- ⑤⑳ Maekawa K, 総 8 名, Inaba M(7 番目): Influence of atherosclerosis on the relationship between anaemia and mortality risk in haemodialysis patients, *Nephrol Dial Transplant* 2008, 23:2329-2336, 査読有
- ⑤⑱ Kurajoh M, Inaba M, Yamada S, Imanishi Y, 他 3 名: Association of increased active PTH(1-84) fraction with decreased GFR and serum Ca in predialysis CRF patients: modulation by serum 25-OH-D, *Osteoporos Int* 2008, 19:709-716, 査読有
- ⑤⑲ Kumeda Y, Inaba M, 他 6 名: Significant correlation of glycated albumin, but not glycated haemoglobin, with arterial stiffening in haemodialysis patients with type 2 diabetes, *Clin Endocrinol*

- (Oxf) 2008, 69:556-561, 査読有
- ⑤6 Ishimura E, 総 10 名, Inaba M(9 番目): Different risk factors for vascular calcification in end-stage renal disease between diabetics and nondiabetics: the respective importance of glycemic and phosphate control, *Kidney Blood Press Res* 2008, 31:10-15, 査読有
- ⑤7 Inaba M, 他 8 名: Increased basal phosphorylation of mitogen-activated protein kinases and reduced responsiveness to inflammatory cytokines in neutrophils from patients with rheumatoid arthritis, *Clin Exp Rheumatol* 2008, 26:52-60, 査読有

[学会発表] (計 8 件)

- ① Nagata Y, Coexpressions of FGF-23 and MEPE in causative tumors of oncogenic osteomalacia, ASBMR 32nd Annual Meeting, 2010/10/16, Metro Toronto Convention Center
- ② Kurajoh M, Reduction of Whole PTH/Intact PTH Ratio is a Predictor of Bone Metabolism by Cinacalcet Treatment in Hemodialysis Patients with Secondary Hyperparathyroidism, ASBMR 32nd Annual Meeting, 2010/10/16, Metro Toronto Convention Center
- ③ Imanishi Y, Suppression of Circulating Fibroblast Growth Factor-23 by Cinacalcet in Patients with Primary Hyperparathyroidism, 12th European Congress of Endocrinology, 2010/4/27, Prague Congress Centre
- ④ Ishii A, Octreotide therapies were unable to improve hypophosphatemia in oncogenic osteomalacia, 14th International Congress of Endocrinology (ICE2010), 2010/3/28, Kyoto International Conference Center
- ⑤ Kurajoh M, Regulation of Circulating Fibroblast Growth Factor-23 by parathyroid hormone in Patients with Primary Hyperparathyroidism, 14th International Congress of Endocrinology (ICE2010), 2010/3/28, Kyoto International Conference Center
- ⑥ Ishii A, Expressions of Somatostatin Receptors on Responsible Tumors are Insufficient for Their Agonist to Normalize Serum Phosphate Levels in Oncogenic Osteomalacia, ASBMR 31th Annual Meeting, 2009/9/14, Colorado Convention Center
- ⑦ Kurajoh M, Cinacalcet Suppresses

Circulating Fibroblast Growth Factor-23 in Patients with Primary Hyperparathyroidism, ASBMR 31th Annual Meeting, 2009/9/13, Colorado Convention Center

- ⑧ Imanishi Y, Activation of Calcium-Sensing Receptor Accelerates Amino-terminal Truncation of Human Parathyroid Hormone (1-84) in the Patients with Primary Hyperparathyroidism, ASBMR 31th Annual Meeting, 2009/9/12, Colorado Convention Center

6. 研究組織

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