Role of Olfactory Marker Protein in Regulating Prolactin Production and Secretion in Lactotrophs

Chan Woo Kang

Department of Medical Science

The Graduate School, Yonsei University

Role of Olfactory Marker Protein in Regulating Prolactin Production and Secretion in Lactotrophs

Chan Woo Kang

Department of Medical Science

The Graduate School, Yonsei University

Role of Olfactory Marker Protein in Regulating Prolactin Production and Secretion in Lactotrophs

Directed by Professor Eun Jig Lee

The Doctoral Dissertation
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy of Medical Science

Chan Woo Kang

December 2018

This certifies that the Doctoral Dissertation of Chan Woo Kang is approved.

Thesis Supervisor : Eun Jig Lee

Thesis Committee Member#1 : Sahng Wook Park

Thesis Committee Member#2 : Yumie Rhee

Thesis Committee Member#3 : Shinae Kang

Thesis Committee Member#4 : Cheol Ryong Ku

The Graduate School
Yonsei University

December 2018

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my advisor Prof. Eun Jig Lee for the continuous support of my Ph.D study and related research, for his patience, motivation, and immense knowledge. He provided me an opportunity to join the team and gave access to the laboratory and research facilities. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D study.

Besides my advisor, I would like to thank the rest of my thesis committee: Prof. Sahng Wook Park, Prof. Yumie Rhee, Prof. Shinae Kang, and Prof. Cheol Ryong Ku for their insightful comments and encouragement, but also for the hard question which incented me to widen my research from various perspectives.

My sincere thanks also go to my fellow laboratory colleagues Dr. Yoon Hee Cho, Dr. Da Ham Kim, Dr. Yang Jong Lee, Ms. Jin Hee Kim, and Mr. Joo Heon Oh.

Last but not the least, I would like to thank my family: my lovely wife, my parents and parents in law, my brother, and to my grandmother for supporting me spiritually throughout writing this thesis and my life in general.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

Role of Olfactory Marker Protein in regulating Prolactin Production and Secretion in Lactotrophs

Chan Woo Kang

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Eun Jig Lee)

Olfactory marker protein (OMP) is a marker of olfactory receptor-mediated chemoreception, even outside the olfactory system. Here, we report that OMP expression in the pituitary gland plays a role in basal and thyrotropin-releasing hormone (TRH)-induced prolactin (PRL) production and secretion. We found that OMP was expressed in human and rodent pituitary glands, especially in PRL-secreting lactotrophs. OMP knockdown in GH4 rat pituitary cells increased PRL production and secretion via extracellular signal-regulated kinase (ERK)1/2 signaling. Real-time PCR analysis and the Ca^{2+} influx assay revealed that OMP was critical for TRH-induced PRL secretion. OMP-knockout mice showed lower fertility than control mice, which was associated with increased basal PRL production via activation of ERK1/2 signaling and reduced TRH-induced PRL secretion. However, both *in vitro* and *in vivo* results indicated that OMP was only required for hormone production and secretion because ERK1/2 activation failed to stimulate cell

proliferation. Additionally, patients with prolactinoma lacked OMP expression in tumor tissues with hyperactivated ERK1/2 signaling. These findings indicate that OMP plays a role in PRL production and secretion in lactotrophs through the modulation of Ca^{2+} and TRH signaling.

Key words : Olfactory marker protein, Prolactin, thyrotropin-releasing hormone, lactotroph

Role of Olfactory Marker Protein in regulating Prolactin Production and Secretion in Lactotrophs

Chan Woo Kang

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Eun Jig Lee)

I. INTRODUCTION

Prolactin (PRL) is a hormone that is mainly secreted by lactotrophs of the anterior pituitary gland and is involved in many biological processes, including reproduction and lactation[1]. The dysregulation of PRL signaling contributes to tumorigenesis—including PRL-secreting adenomas or prolactinomas—leading to pathological hyperprolactinemia. In addition, PRL hypersecretion causes hypogonadism and infertility[2].

PRL secretion is controlled by multiple factors. Dopamine, secreted by hypothalamic neurons, is the major inhibitor of pituitary PRL secretion[3], which is induced by thyrotropin-releasing hormone (TRH) and estrogen (E2). TRH is secreted by the hypothalamus and transported to the pituitary gland via circulation to stimulate PRL synthesis and secretion, although the underlying mechanisms are not fully understood[4].

Olfactory marker protein (OMP) is a small, cytoplasmic protein that is abundantly and almost exclusively expressed in vertebrate olfactory neurons[5-8]. Previous studies have shown that OMP modulates olfactory signal transduction in part by participating in Ca^{2+} clearance[8-13]. Microarray and RNA sequencing analyses have revealed that OMP is also expressed in non-olfactory tissues, often with odorant receptors (ORs), which constitute a major class of G protein-coupled receptor (GPCR)[14,15]. However, it is not known whether OMP function is conserved across tissues. Recent studies have suggested a link between OMP and the endocrine system, especially in neuroendocrine neoplasia and hormone secretion[15-18]. However, to date, there have been no studies investigating the role of OMP in the functioning of the pituitary gland, which is considered the master regulator of the neuroendocrine system. To address this issue, the present study investigated OMP expression in the mouse and human pituitary gland and characterized its mechanism of action in pituitary lactotrophs.

II. MATERIALS AND METHODS

1. Plasmid constructs and transfection

Plasmids expressing OMP were purchased from Addgene (Cambridge, MA, USA). GH4 cells were seeded at a density of 0.5×10^6 cells/60-mm dish, 1 day before transfection. The cells were transfected with the appropriate expression plasmids using the Polyjet transfection reagent (SignaGen, Rockville, MD, USA), and cultured at 37 ℃ for 24 hr, followed by treatment with TRH or saline for an additional 30 min prior to lysis.

2. Cell culture

Rat pituitary cell lines, GH3 and GH4, were purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA) and 1% Pen/Strep (Hyclone). Cells were cultured in a humidified tissue culture incubator at 37 ℃ in an atmosphere of 5% CO_2.

3. Tissue culture

Pituitary glands were isolated from 20-week old C57BL6 OMP-WT or -KO mice (n = 5). The anterior pituitary glands were rapidly removed and processed for explant cultures. All procedures were carried out in a laminar-flow hood. After aseptically trimming adhering tissue residues, the pituitary tissue was transferred to a sterile conical tube and washed with cold HEPES-buffered salt solution (HBSS) buffer. The tissue explants were individually placed on 40-mesh Millicell cell culture inserts (Millipore, Billerica, MA, USA) in 1 ml Ham's F-10 culture medium supplemented with 10% FBS and antibiotics in plastic culture dishes. The cultures were maintained for up to 1 week under controlled conditions (humidified atmosphere, 37 ℃, 5% CO_2 in air) and the medium was changed daily.

4. RNA isolation and real-time PCR analysis

Total RNA was extracted from GH3 or GH4 cells or mouse pituitary tissue lysates using Isol-RNA lysis reagent (5 PRIME, Hilden, Germany), and cDNA was prepared using ReverTra Ace (Toyobo, Osaka, Japan). The following forward and reverse primers were used for amplification.

GAPDH 5′-GGATGGAATTGTGAGGGAGA-3′ (sense) and 5′-GAGGACCAGGTTGTCTCCTG-3′ (antisense); PRL, 5′-CATCAATGACTGCCCCACTTC-3′ (sense) and 5′-CCAAACTGAGGATCAGGTTCAAA-3′ (antisense); mouse OMP, 5′-CGTCTACCGCCTCGATTTCA-3′ (sense) and 5′-CAGAGGCCTTTAGGTTGGCA-3′ (antisense); rat OMP, 5′-GCAGTTCGATCACTGGAACG-3′ (sense) and 5′-ATCCATGGCATCGGAGTCTTC-3′ (antisense); TRHR1, 5′-CATGTTCAATAACGGCCTTTACC-3′ (sense) and 5′-GGGCTGGAGAGAAATGAGTTGACA-3′(antisense); Golf 5′-TACCAGCTGATTGACTGTGC -3′ (sense) and 5’-TTGCATATTCTGGGAAATAG-3’ (antisense); and Adenylyl cyclase III 5’-CGGTGGAGAAGGAGAAGCAGAGTGG-3’ (sense) and 5’-CCTCCGTTTCCATCCCTGCCGTTGC-3’ (antisense). Quantitative real-time PCR reactions were performed on the Step One Plus using SYBR-based detection reagents (Applied Biosystems, CA, USA).

5. Western blotting

Whole cell protein lysates were prepared, and the western blot assay was performed according to standard procedures. Briefly, cells were chilled on ice, washed twice with ice-cold phosphate-buffered saline, and lysed in buffer containing 1 mM phenylmethylsulfonyl fluoride and 1× protease inhibitors

(Sigma-Aldrich). Protein concentrations were determined with the Bradford assay kit (Bio-Rad, Hercules, CA, USA). Equal amounts of protein in cell lysates were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a membrane that was incubated with the primary antibody, overnight at 4 ℃; the primary antibodies used were rabbit anti-phospho-ERK1/2 (T202/Y204), mouse anti-ERK1/2, and rabbit anti-phospho-PKCα/β (T638/641) (Cell Signaling Tec, MA, USA). Rabbit anti-OMP, goat anti-PRL, and mouse anti-β-actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Blots were washed three times with TBST (Tris-buffered saline containing 0.05% Tween 20), and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 1 hr at 25 ℃. Secondary antibodies were donkey anti-rabbit IgG-HRP antibody (1:5,000; Santa Cruz), donkey anti-mouse IgG-HRP antibody (1:5,000; Santa Cruz), or donkey anti-goat IgG-HRP antibody (1:5,000; Santa Cruz). Immunoreactivity was detected with the SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, MA, USA). The intensity of protein bands was quantified using ImageJ and normalized to that of β-actin in each sample.

6. Immunofluorescence analysis

Paraffin-embedded rat pituitary samples were cut into 4-μm sections that were deparaffinized in xylene and rehydrated in a graded series of ethanol. Antigen retrieval was carried out in 10 mM sodium citrate buffer (pH 6.0). Sections were blocked in 5% normal serum for 1 hr. Incubation with specific antibodies for rabbit anti-OMP (1:100, Santa Cruz, CA, USA), goat anti-GH (1:200), goat anti-PRL (1:200, Santa Cruz, CA, USA), mouse anti-ACTH (1:200, Santa Cruz, CA, USA), mouse anti-TSH (1:200, Santa Cruz, CA, USA), and mouse anti-FSH (1:200, Santa Cruz, CA, USA) was performed overnight at 4 ℃. After washing with TBST, secondary antibodies, donkey anti-rabbit-FITC, donkey anti-goat Cy3, or donkey anti-mouse Cy3 (1:200; Jackson ImmunoResearch, West Grove, PA,

USA), were added, and the sections were incubated for 2 hr at room temperature. After washing, the sections were mounted with Vectashield medium (Vector Laboratories, Burlingame, CA, USA). Samples were visualized with an Axioskop microscope (Carl Zeiss).

For quantification of colocalization of cells expressing the Cy3-OMP and FITC-hormone, ImageJ software was used to calculate a Pearson's coefficient, based on the correlation between Red and Green signal overlap in at least 3 different normal human pituitary sections.

7. siRNA transfection

siRNA targeting OMP (siOMP-GCA GUU CGA UCA CUG GAA CGU GGU U) was synthesized by Invitrogen and transfected into cells using Lipofectamine RNAiMAX (Invitrogen).

8. Cell cycle analysis

For the cell-cycle assay, 4.0×10^5 cells were fixed with ice cold 70% ethanol for 1 hr on ice. The cells were then centrifuged at 800 rpm for 5 min, followed by resuspension in 1 mL of PI Master Mix containing 40 μL of propidium iodide (Invitrogen), 10 μL of RNase A (Sigma-Aldrich), and 950 μL of PBS. After incubation at 37 ℃ for 30 min, DNA ploidy was analyzed by flow cytometry.

9. Cell proliferation assay

MTS assays were performed using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit (Promega). To measure cell proliferation, siCON- or siOMP-treated cells were seeded into 96-well plates (3000 cells/well). Then, cell proliferation was measured every 24 hr for 5 days. Briefly, 20 μL of MTS labeling reagent was added to each well and incubated at 37 ℃ for 1 hr. Absorbance was measured at 490 nm. The relative proliferation of siOMP-treated cells was normalized to that of siCON-treated cells after background subtraction.

10. Animals

Male OMP knock out (−/−) transgenic mice were obtained from the Jackson Laboratory (B6;129P2-Omptm3Mom/MomJ). WT littermates served as controls. Mice were maintained under controlled conditions (12:12-hr light/dark cycle, 21℃) with free access to laboratory chow and tap water. All animal experiments were performed according to the applicable Korean laws, reviewed and approved by the Institutional Animal Care and Use Committee of the Yonsei University Severance Hospital, Seoul, Korea (IACUC Approval No: 2015-0025), and carried out in accordance with the approved guidelines by the IACUC.

Mice were genotyped by PCR using genomic DNA isolated from cut tails. The presence or absence of OMP was determined by multiplex PCR using primers OMP137, OMP138, and OMP139, according to genotyping conditions provided by the Jackson Laboratory (https://www.jax.org/strain/006667).

11. Fura-2 Ca^{2+} assay

Cells were grown to 100% confluence in 96-well plates and washed with HBSS. They were then incubated in HBSS containing 3 μM Fura-2 AM (Invitrogen) at 37 ℃ for 30 min. Cells were washed thrice and incubated in HBSS at room temperature for 20 min to allow dye de-esterification. Fluorescence was detected every 5 s with a Gen 5 Luminescence spectrometer (BioTek, Winooski, VT, USA) at 340 and 380 nm (excitation) and 510 nm (emission). TRH (Sigma-Aldrich, St. Louis, MO, USA) was prepared in HBSS immediately before use. Fluorescence values are reported as F/F0, which was calculated using the following formula: $[\Delta F = (340\ nm)f/(380\ nm)f - (340\ nm)_0/(380\ nm)_0]$.

12. ELISA for PRL

GH4 cells (1×10^6) were seeded in a 6-well plate (SPL Life Sciences, Pocheon, Korea) and cultured for 18 hr. After two washes in a saline solution, the cells were

incubated in a fresh culture medium with saline or TRH for 30 min. The medium was transferred to Eppendorf tubes that were centrifuged for 3 min at 700 ×g. A 100 μl aliquot of supernatant was collected to determine the PRL concentration in the medium (ng/ml) using a mouse/rat ELISA kit (Calbiotech). OMP-WT and OMP-KO mouse plasma samples were collected and frozen until use.

13. Patients and samples

Human prolactinomas were derived from the Yonsei Pituitary Tumor Center (Seoul, South Korea). Briefly, human prolactinomas (n = 3, three female) were obtained during transsphenoidal surgery as part of an ongoing accession of human pituitary tumors. Tumors were frozen in liquid nitrogen and stored at -80 ℃ until use. Clinicopathological parameters were retrospectively collected from our institution. Normal pituitary glands (n = 3, three female) were obtained from the National Forensic Service (Gangwon-do, South Korea). The study was approved by the Institutional Review Board of Yonsei University, and informed consent was obtained for all subjects (IRB number: 4-2011-0740).

14. Immunohistochemistry

Human samples were fixed overnight in formalin. Four-micron thick tissue sections were cut from each block to perform either hematoxylin-eosin or immunostaining with OMP, and PRL antibodies. After dewaxing and rehydrating paraffin sections and antigen retrieval by pretreatment with high temperature at pH 6 citrate. After antigen retrieval, tissue sections were immunolabeled with primaries antibodies used as follows: rabbit anti-OMP (1:200, Santa Cruz, CA, USA), goat anti-PRL (1:500, Santa Cruz, CA, USA). Subsequently, tissues were incubated with secondary antibody polymer for 10 min (Leica Microsystems, Germany) and developed with DAB-chromogen for 10minutes. All slides were counterstained with hematoxylin.

15. Statistical analysis

Statistical analyses were carried out using Prism software v.4.0.0 (GraphPad Inc., La Jolla, CA, USA). Each experiment was repeated at least thrice. Statistical significance was determined using one-way ANOVA followed by post-hoc Tukey analysis, and Student's t-test to compare the means of 2 different groups. Significant differences are indicated with asterisks (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

III. RESULTS

1. Lactotroph-specific expression of OMP in pituitary gland

To determine whether OMP is expressed in the anterior pituitary gland, we carried out an immunohistochemical analysis using antibodies against various hormones, including PRL, growth hormone (GH), adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), and follicle-stimulating hormone (FSH) produced by somatotrophs, lactotrophs, adrenocorticotrophs, thyrotrophs, and gonadotrophs, respectively, of the human pituitary gland. We observed OMP-expressing cells significantly colocalized with PRL-expressing cells. In contrast, a portion of cells positive for TSH, FSH, GH, and ACTH was negative for OMP (Figure 1A). Consistent with these findings, rat pituitary immunolabeling revealed a high degree of PRL and OMP co-expression (Figure 1B). We also examined OMP expression in GH3 and GH4 PRL-secreting rat pituitary cells by western blotting. OMP levels were found to be similar to those in the olfactory bulb (Figure 1C), with a higher level observed in GH4 than in GH3 cells. These findings suggested that OMP plays an important role in pituitary lactotrophs.

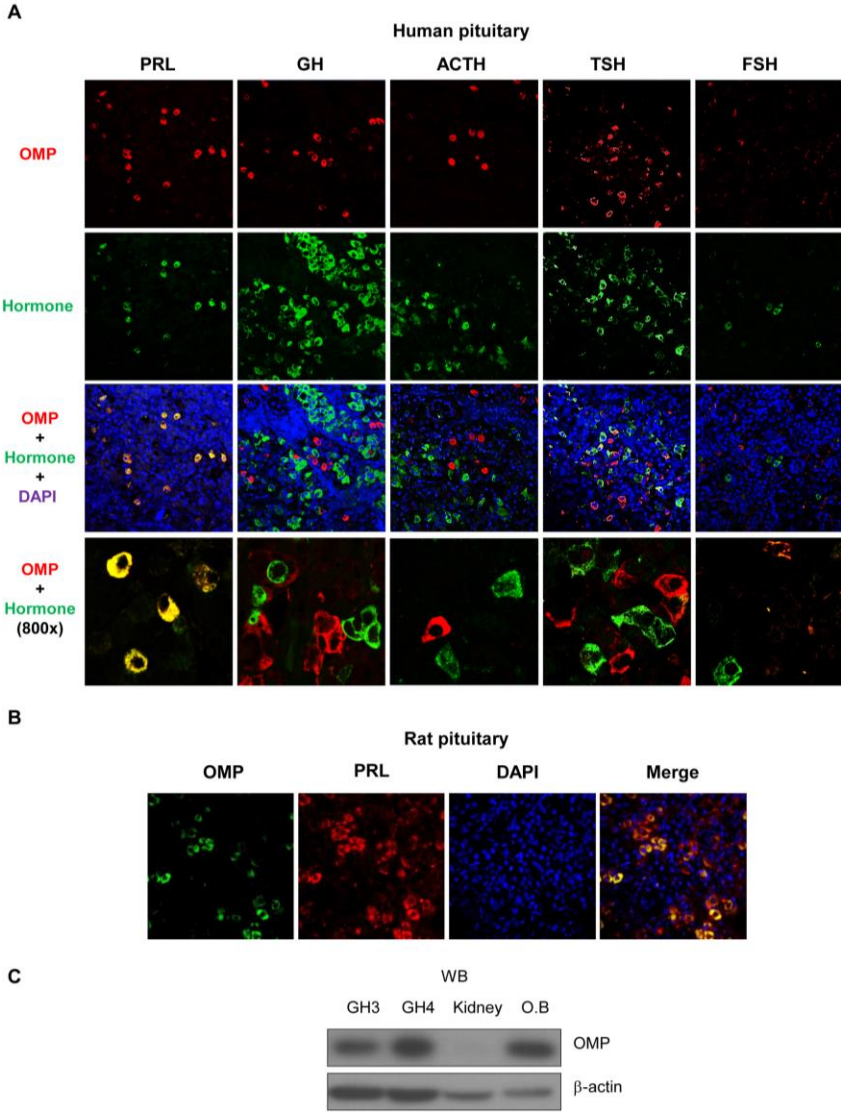

Figure 1. Lactotroph-specific expression of OMP in pituitary gland. (A) Representative images of OMP immunofluorescence labeling in normal human pituitary sections. OMP expression and all five cell types in the anterior pituitary were detected by antibodies against PRL, GH, ACTH, TSH, and FSH. OMP co-localized with PRL in lactotrophs, but not in other cell types. Images were obtained with a laser scanning microscope at 200× and 800× magnification. (B) Representative images of OMP and PRL immunofluorescence labeling in rat

pituitary sections. (C) OMP expression in rat PRL-secreting cell lines. OMP protein levels in GH3 and GH4 cells, kidney (negative control), and olfactory bulb (positive control) was determined by western blotting. Blots are representative of three experiments.

2. Effects of OMP knockdown and overexpression in PRL synthesis, secretion and related pathways in GH4 cell line

As OMP was found to be expressed in human and rat lactotrophs, we investigated whether modulating its expression would affect PRL secretion. We used short interfering (si)RNAs to knock down OMP expression in GH4 cells and analyzed the levels of secreted PRL using enzyme-linked immunosorbent assay (ELISA). PRL levels were 2.15-fold higher in the culture supernatant of GH4 cells transfected with siOMP (siOMP-GH4 cells) than in control siRNA-transfected cells (siCON-GH4) (Figure 2A). We examined the association between OMP expression and PRL synthesis using real-time PCR (RT-)PCR and found that PRL mRNA levels were 2.01-fold higher in siOMP-GH4 than in siCON-GH4 cells (Figure 2B). A similar trend was observed for PRL protein expression. Extracellular signal-regulated kinase (ERK1/2) signaling is a point of convergence for PRL gene transcription[19]; a western blot analysis also revealed an increase in ERK1/2 phosphorylation in siOMP-GH4 cells, compared to that in the control cells (Figure 2C).

We also investigated whether OMP overexpression in lactotrophs could alter PRL gene expression. The overexpression of OMP in GH4 (OMP-GH4) reduced PRL secretion and PRL synthesis compared to the control group (Figure 2D and 2E). Moreover, ERK1/2 phosphorylation was decreased in OMP-GH4 cells, compared to Empty-GH4 cells (Figure 2F). These data indicated that OMP inhibits PRL synthesis by suppressing ERK1/2 phosphorylation.

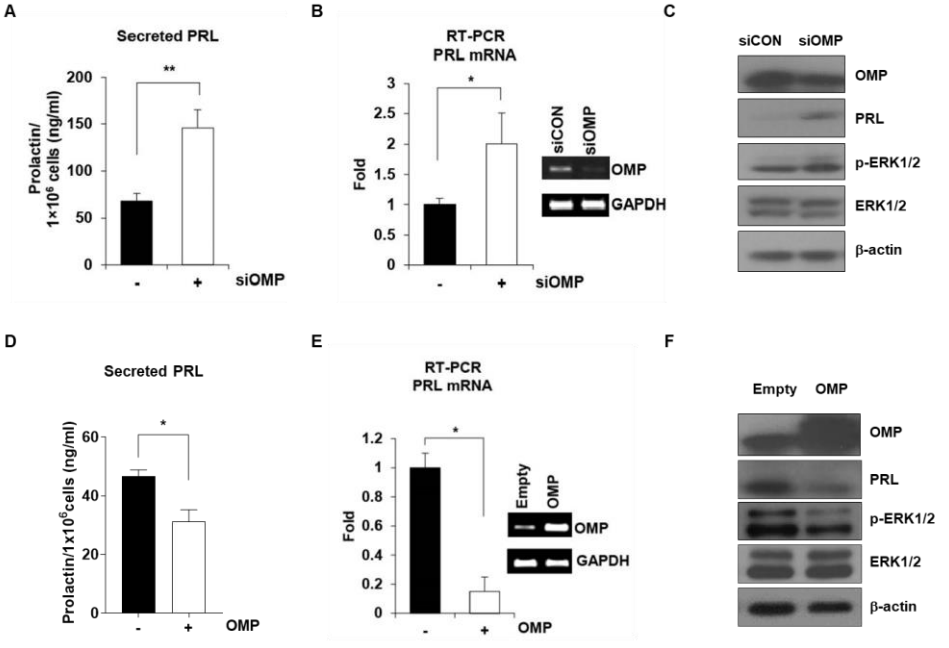

Figure 2. Effects of OMP knockdown and overexpression in PRL synthesis, secretion and related pathways in GH4 cell line. (A) PRL concentration in the supernatant of GH4 cells transfected with siCON or siOMP was evaluated using ELISA. Results represent the mean of at least three independent experiments. **P < 0.01 vs. siCON group. (B) RT-PCR analysis of PRL mRNA expression in GH4 cells transfected with siCON (−) or siOMP (+). *P < 0.05 vs. siCON group. (C) Effect of siCON or siOMP on the expression of indicated proteins in GH4 cells, as determined by western blotting. (D) PRL concentration in the supernatant of GH4 cells transfected with empty vector (−) or OMP overexpression plasmid (+) was evaluated using ELISA and (E) RT-PCR analysis of PRL mRNA expression in GH4 cells transfected with empty vector (−) or OMP overexpression plasmid (+). Data represent mean ± SE of triplicate samples. *P < 0.05 vs. empty vector group. (F) Expression of indicated proteins in GH4 cells transfected with empty vector of OMP overexpression plasmid, as determined by western blotting.

3. Loss of OMP is not associated with lactotroph hyperplasia in vitro and in vivo

The mitogen-activated protein kinase (MAPK) pathway is a conserved developmental pathway that regulates organ development and tissue homeostasis by transmitting signals. Activation of ERK1/2 triggers the transcriptional activation of multiple target genes involved in modulating the cellular processes of differentiation, proliferation, survival, migration, and angiogenesis[20]. In addition, there are preclinical data and human pituitary tumor studies that are compatible with increased ERK1/2 pathway activity in pituitary tumors[21]. As OMP knockdown increased ERK/12 phosphorylation, we investigated whether aberrant OMP-induced ERK1/2 activation could contribute to the development of pituitary hyperplasia or tumors. However, cell cycle distribution and cell proliferation were unaltered in siOMP-GH4 cells (Figure 3A and B). To further explore this finding, we compared the pituitary gland size and weight of OMP KO mice with those from OMP WT mice. Consistent with siOMP-GH4 cells, pituitary gland size and weight were comparable between OMP WT and OMP KO mice (Figure 3C, and D). These findings demonstrated that ERK1/2 activation induced by OMP deficiency promotes PRL production and secretion but not pituitary hyperplasia.

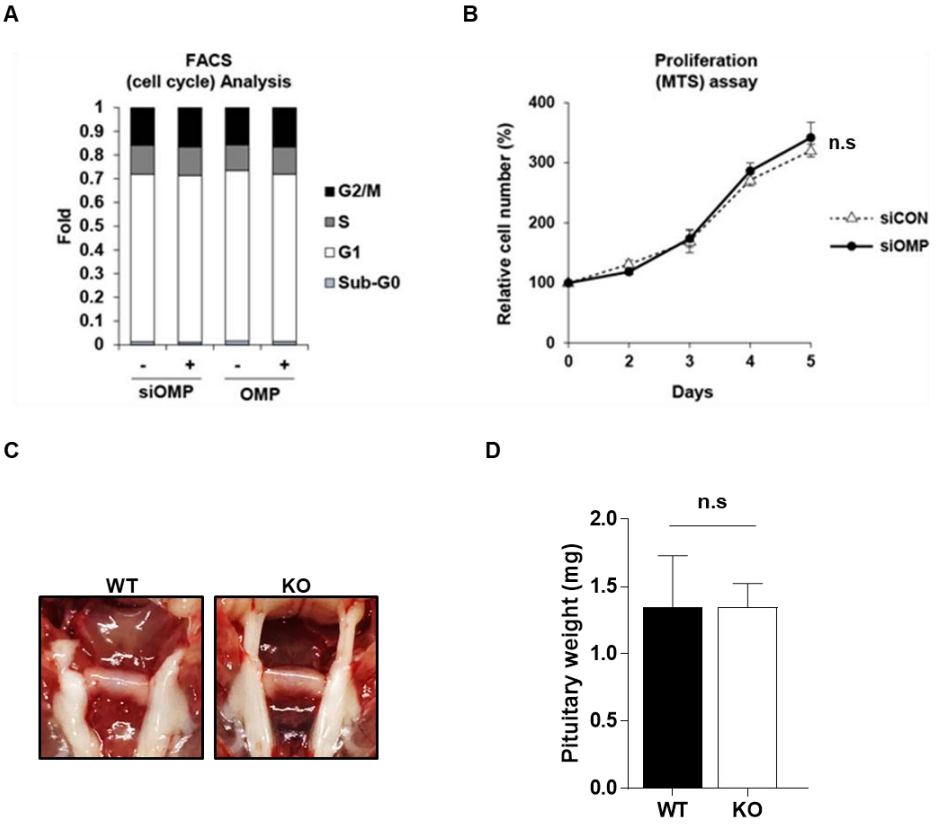

Figure 3. Loss of OMP is not associated with lactotroph hyperplasia *in vitro* and *in vivo*. (A) GH4 cells were transfected with siCON or siOMP for 48 hr, and cell cycle distribution was determined by propidium iodide staining and fluorescence-activated cell sorting. (B) Proliferation of GH4 cells transfected with siCON or siOMP for 5 days. Data are representative of three experiments. (C) Gross morphology of pituitary glands. Representative glands from adult male OMP WT (WT) and OMP KO (KO) mice are shown (30-week-old). (D) Total weight of pituitary glands (30-week-old males, n=3 per group). ns, Not significant; vs. OMP WT (WT).

4. OMP modulates TRH-induced ERK/12 phosphorylation and Ca^{2+} influx

In pituitary lactotrophs, PRL gene expression is stimulated by neuropeptides such as TRH and E2, and is suppressed by dopamine via D2-type receptors [22-27]. Based on our finding that OMP could modulate PRL synthesis and secretion in pituitary lactotrophs, we investigated the signaling pathway involved. GH4 cells transfected with either siCON or siOMP were treated with the dopamine agonist cabergoline (Cab, 1 μM), β-estradiol (E2, 1 nM), or TRH (100 nM). There was no significant difference in PRL mRNA levels between siCON-GH4 and siOMP-GH4 cells following treatment with Cab or E2, as determined by real-time PCR (Figure 4). However, PRL expression was increased by 2.46-fold in siCON-GH4 cells by TRH treatment, whereas no change was observed in siOMP-GH4 cells (Figure 4). In addition, TRH treatment increased PRL secretion, whereas OMP knockdown abolished this effect (Figure 5A). Moreover, ERK1/2 phosphorylation increased by about 4.82-fold in siCON-GH4 cells upon TRH treatment (Figure 5B). ERK1/2 phosphorylation levels were similarly increased in siOMP-GH4 cells with or without TRH treatment (Figure 5B). These findings indicate that OMP inhibits TRH-induced ERK1/2 phosphorylation and PRL secretion.

In lactotrophs, TRH acts via a GPCR to increase phospholipase C activity, Ca^{2+} release from intracellular stores, and Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels[28]. Based on these findings and the observations that basal PRL synthesis and secretion and TRH insensitivity were increased by blocking OMP expression in GH4 cells, we examined whether Ca^{2+} influx was also altered using the Fura-2AM Ca^{2+} influx assay. Consistent with previous studies[28], treatment with 100 nM TRH markedly increased intracellular Ca^{2+} levels in siCON-GH4 cells, but not in siOMP-GH4 cells (Figure 6A). Moreover, basal intracellular Ca^{2+} levels were 1.33-fold higher in siOMP-GH4 cells than in siCON-GH4 cells (Figure 6B). These data indicate that OMP regulates basal intracellular Ca^{2+} levels and TRH-induced PRL exocytosis.

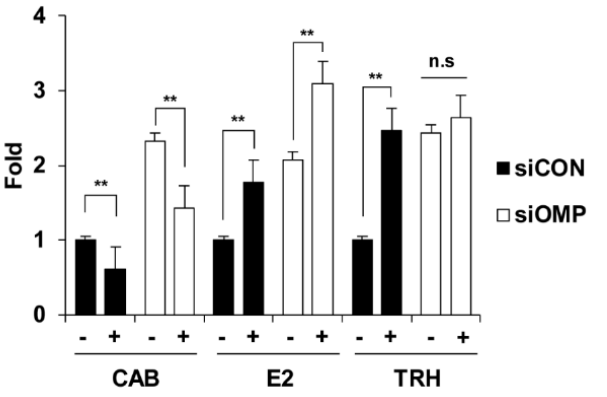

Figure 4. OMP modulates TRH-induced PRL synthesis in GH4 cells. RT-PCR analysis of PRL mRNA expression in GH4 cells transfected with siCON or siOMP and treated with Cab (1 μM), E2 (10 nM), or TRH (100 nM). Results represent the mean of at least three independent experiments. ns, Not significant; **, $P < 0.01$ vs. non-transfected (NT) control.

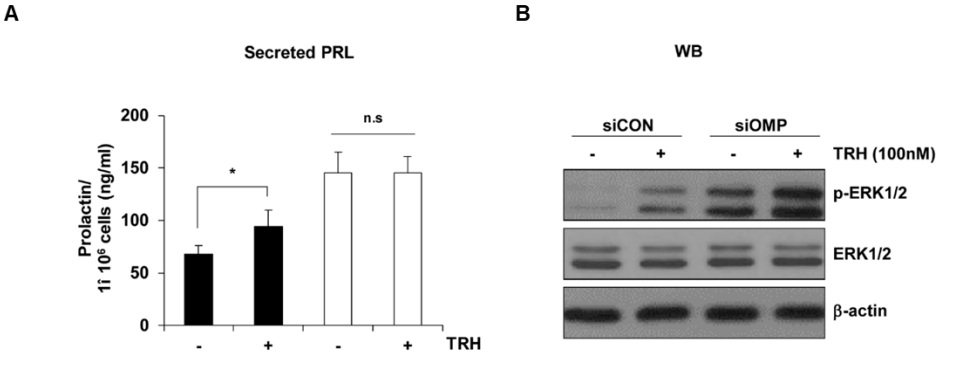

Figure 5. OMP modulates TRH-induced PRL secretion and ERK1/2 phosphorylation in GH4 cells. (A) PRL secretion by GH4 cells transfected with siCON or siOMP. Results represent the mean of at least three independent experiments. ns, Not significant; *, $P < 0.05$ vs. NT control. (B) GH4 cells were transfected with indicated siRNAs and phosphate-buffered saline (PBS) or 100 nM TRH was added for 10 min, followed by western blotting.

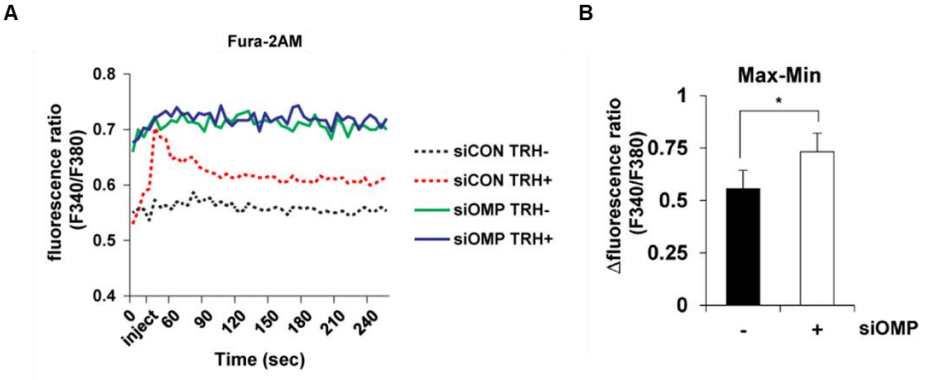

Figure 6. OMP modulates Ca^{2+} in GH4 cells. (A) GH4 cells were transfected with siCON or siOMP, then loaded with Fura 2-AM and treated with TRH (100 nM) to stimulate Ca^{2+} release. Intracellular Ca^{2+} levels were measured based on ratiometric measurements of absorbance at 340 and 380 nm (340/380). Intracellular Ca^{2+} levels were continuously monitored for 5 min. Values represent means. (B) Peak store-operated Ca^{2+} entry in PBS-treated siCON- and siOMP-transfected cells. Values represent mean ± SE. *, $P < 0.05$ PRL secretion by GH4 cells transfected with siCON or siOMP. Results represent the mean of at least three independent experiments. ns, Not significant; *, $P < 0.05$ vs. NT control.

5. Loss of OMP expression leads to elevated basal PRL levels and loss of sensitivity to TRH in vivo

We next examined whether OMP expression in pituitary lactotrophs plays a role in basal PRL secretion and TRH sensitivity in vivo using OMP-knockout (OMP-KO) mice[5,29]. We evaluated circulating PRL levels in 20-week old male OMP-wild type (OMP-WT), OMP-heterozygote (OMP-het), and OMP-KO mice using ELISA, and found that basal circulating PRL levels were higher in OMP-KO than in OMP-WT mice (11.9 ± 2.3 ng/ml vs. 5.6 ± 0.6 ng/ml) (Figure 7).

To establish the function of OMP in anterior pituitary tissue, we examined ERK1/2 activation in primary tissue cultures. Consistent with the observed increase in circulating PRL concentration, OMP-KO mice showed a higher basal ERK1/2 phosphorylation level than OMP-WT mice. Anterior pituitary specimens from OMP-WT mice showed increased ERK1/2 phosphorylation upon TRH treatment, compared to those from saline-treated OMP-WT mice, whereas in OMP-KO mice, ERK1/2 phosphorylation levels were unchanged by TRH treatment (Figure 8A).

To evaluate TRH-induced PRL secretion in the anterior pituitary, PRL levels in the supernatant of anterior pituitary tissue cultures were estimated using ELISA. Consistent with the results of the western blot, secreted PRL levels were 2.35-fold higher in cultures of OMP-WT anterior pituitary tissue treated with TRH than in those treated with saline (9.7 ± 0.9 vs. 4.13 ± 0.6 ng/ml). However, in the absence of OMP, secreted PRL levels were comparable between saline- and TRH-treated samples (8.24 ± 1.6 and 8.53 ± 1.7 ng/ml, respectively), indicating that the loss of OMP resulted in TRH desensitization (Figure 8B). These data suggest that OMP mediates pituitary lactotroph PRL synthesis and TRH-induced PRL secretion.

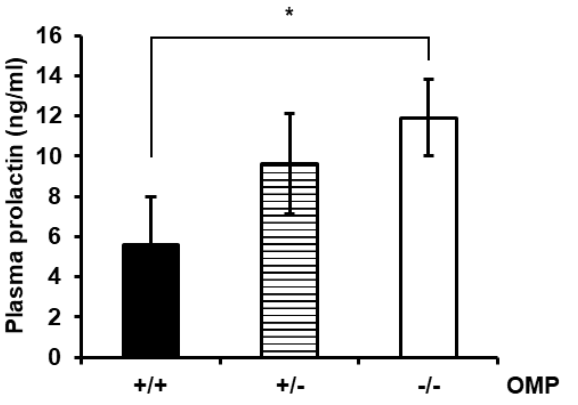

Figure 7. Loss of OMP expression leads to elevated basal PRL levels *in vivo*. Basal circulating PRL levels in OMP-WT (+/+), OMP heterozygote (+/−), and OMP-KO (−/−) mice, as determined by ELISA. Values represent the mean ± SE (OMP+/+, n = 6; OMP+/-, n=6; OMP-/- n=6). *, P < 0.05 vs. +/+ group.

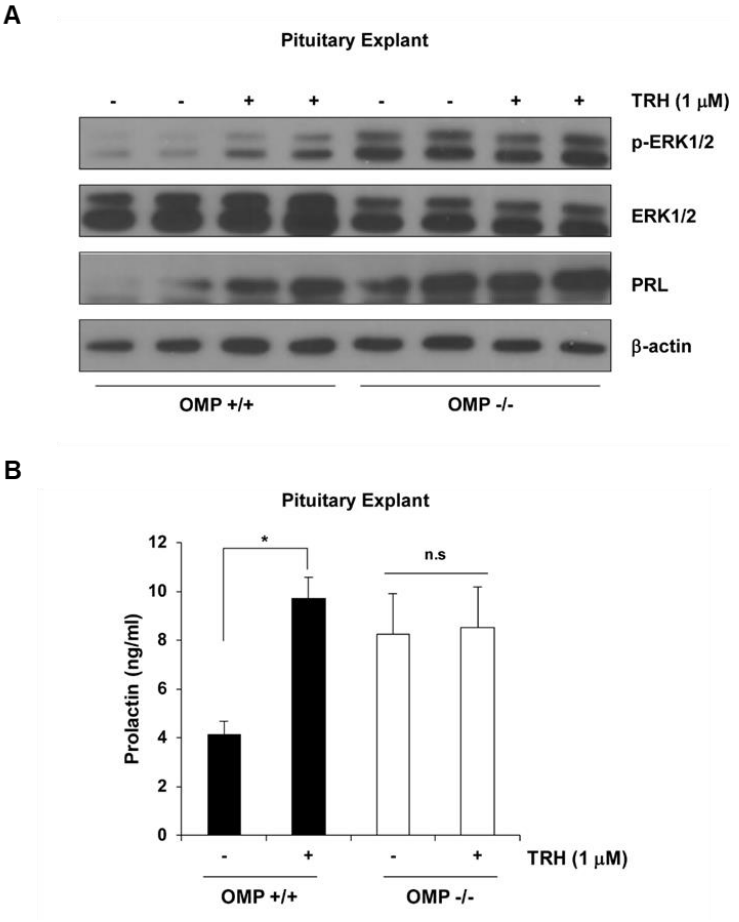

Figure 8. Loss of OMP expression leads desensitization of TRH *in vivo*. (A) ERK1/2 phosphorylation in OMP-WT (+/+) or OMP-KO (−/−) anterior pituitary tissue treated with saline or TRH (1 μM), as determined by western blotting. β-Actin served as a loading control. (B) PRL levels in the culture supernatant of OMP-WT (+/+) and OMP-KO (−/−) anterior pituitary tissue treated with saline or TRH (1 μM), as determined by ELISA. Results represent the mean of at least three independent experiments. ns, Not significant; *, $P < 0.05$ vs. (−) control.

6. OMP expression is dysregulated in PRL-secreting pituitary adenoma

To confirm the regulation of PRL by OMP in the human pituitary, we analyzed OMP expression in normal pituitary and PRL-secreting adenoma (prolactinoma) tissue samples by western blotting. Consistent with results obtained in cell cultures and mice, protein kinase (PK)C and ERK1/2 phosphorylation levels, as well as total ERK1/2 and PRL expression, were higher in prolactinoma than in normal pituitary tissues. In contrast, relative OMP expression was lower in the former than in the latter (Figure 9). An immunofluorescence (IF) analysis revealed that OMP was expressed in PRL-secreting cells of normal human pituitary tissue but was almost undetectable in adenoma tissue. OMP expression was particularly low in areas of high PRL immunoreactivity (Figure 10A). Next, we performed immunohistochemistry in the serial sections to investigate how OMP expression differs from normal and prolactinoma tissues. In line with IF results, OMP was expressed only in the lactotrophs in normal pituitary tissues. However, OMP expression was undetectable in tumor part of prolactinoma tissues, which express high level of PRL. Still, OMP was expressed in the normal tissue adjacent to the tumor in the prolactinoma tissues (Figure 10B). These results suggest that aberrant OMP expression in lactotrophs increase ERK1/2 activation and PRL secretion, thereby promoting prolactinoma and tumorigenesis.

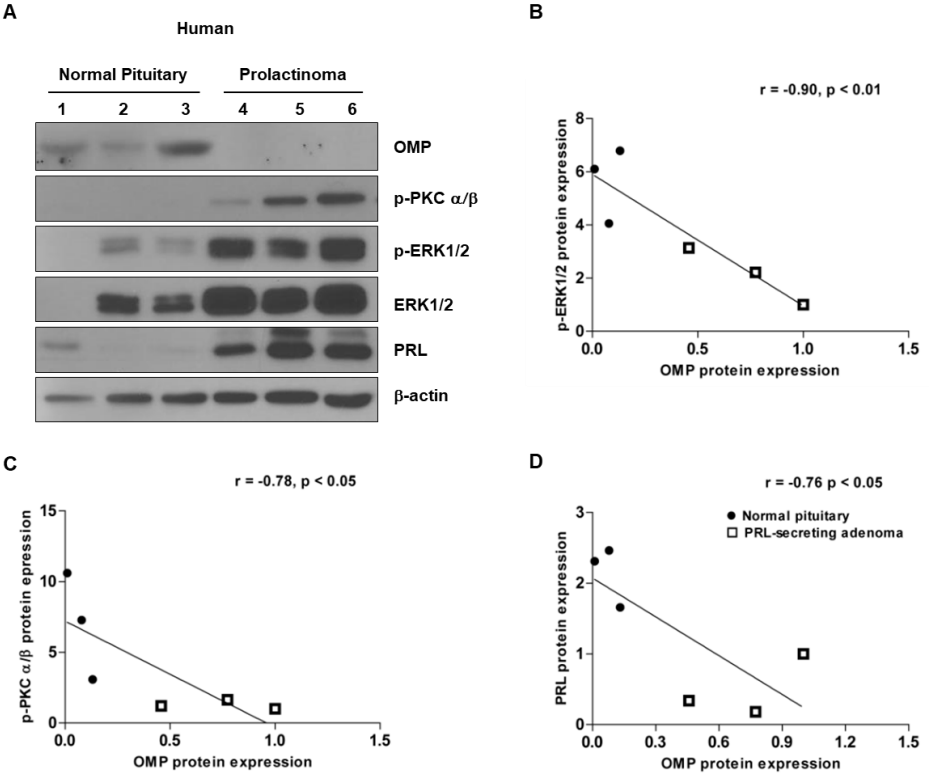

Figure 9. Negative correlation of expression between OMP and PKC-ERK1/2 in prolactinoma patients. (A) Negative correlation between OMP and PKC-ERK1/2 levels in normal human pituitary (lanes 1–3) and prolactinoma (lanes 4–6) tissues, as determined by western blotting. β-actin served as a loading control. (B-D) OMP expression is dysregulated in PRL-secreting pituitary adenoma. Western blot analysis in (A) was analyzed by Pearson's correlation coefficients and their statistical significance are indicated in panels. (B) Negative correlation between OMP protein expression levels and PRL protein expression (Pearson r = -0.76, P < 0.05). (C) Negative correlation between OMP protein expression levels and p-PKC α/β protein expression (Pearson r = -0.78, P < 0.05). (D) Negative correlation between OMP protein expression levels and p-ERK1/2 protein expression (Pearson r = -0.90, P < 0.01).

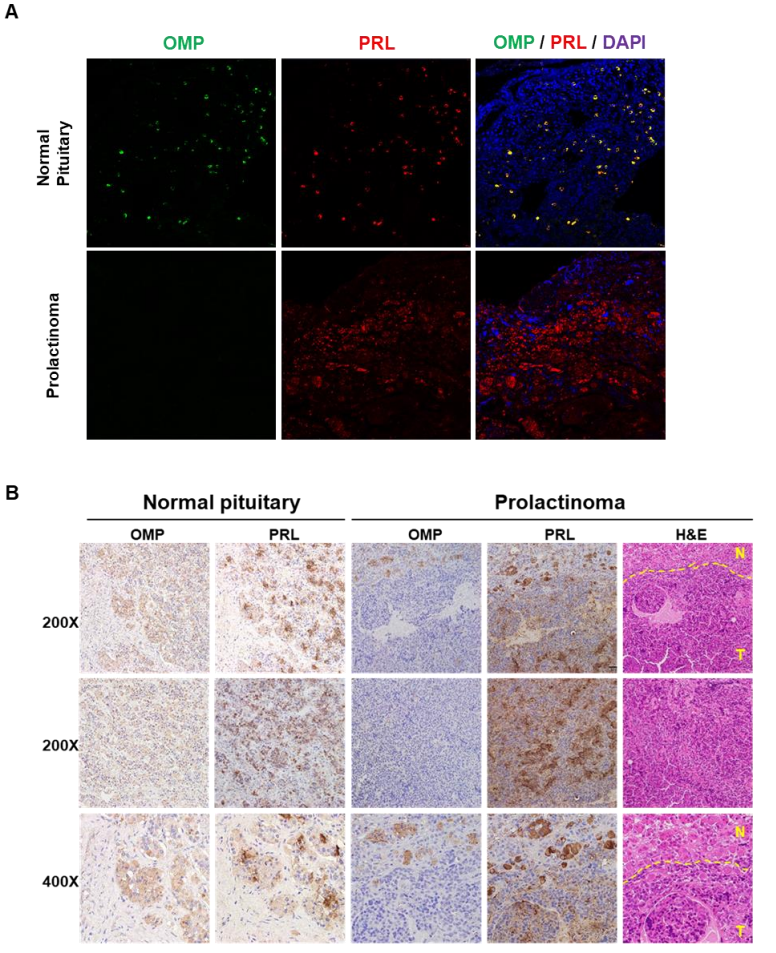

Figure 10. OMP expression is dysregulated in prolactinoma patients. (A) Representative images of OMP (green) and PRL (red) immunofluorescence labeling in normal and prolactinoma tissue sections. Images were obtained by laser scanning microscopy at 100× magnification. (B) Representative images of OMP and PRL immunostaining in normal and prolactinoma sections. Representative hematoxylin and eosin (H&E) staining of prolactinoma clearly show the difference between normal (N) and tumor (T) tissue.

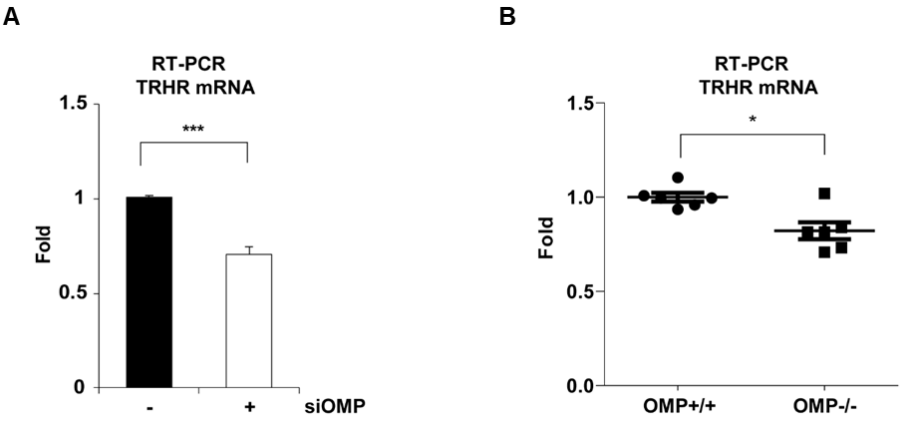

Figure 11. TRHR mRNA expression in GH4 cells and OMP KO mice. (A) RT-PCR analysis of thyrotropin-releasing hormone receptor (TRHR) mRNA expression in GH4 cells transfected with siCON or siOMP. Results represent the mean of at least three independent experiments. ***, $P < 0.001$ vs. siCON-GH4. (B) RT-PCR analysis of TRHR mRNA expression in OMP-WT (+/+) and OMP-KO (−/−) mice. Results represent the mean of at least three independent experiments. *, $P < 0.05$ vs. OMP+/+.

IV. DISCUSSION

The results of this study indicate that OMP is involved in PRL production and secretion by lactotrophs. OMP deficiency increased PRL expression and release *in vitro* and *in vivo* by failing to suppress ERK1/2 phosphorylation and modulate intracellular Ca^{2+} levels. In addition, OMP was found to be associated with PRL secretion induced by TRH, but not by other known neurohormones such as dopamine and E2. Finally, OMP expression was negatively correlated with PKC-ERK1/2 activation in human pituitary gland.

Recent studies have reported that OMP and ORs are expressed in non-olfactory tissues, including the pancreas, colon, bladder, and thyroid gland[15,17,30-32]. However, there have been no previous investigations on the expression and physiological functions of OMP in the pituitary gland. In the present study, we provide the first evidence that OMP is co-expressed with PRL in lactotrophs. Moreover, the higher expression of OMP in GH4 than in GH3 cells suggested its involvement in PRL regulation (Figure 1), as GH4 cells are derived from GH3 cells and exhibit many features of lactotrophs[33-35].

ERK1/2 activation plays an important role in the proliferation of PRL-secreting adenomas and in PRL secretion by the pituitary gland[36,37]. ERK1/2 phosphorylation was increased by OMP deficiency (Figure 2, 8), which was associated with increased PRL secretion, but did not alter cell proliferation or induce tumorigenesis (Figure 3). This was consistent with a previous study, which demonstrated that the activation of MAPK in GH4 cells induced the differentiation of bihormonal somatolactotroph GH4 precursor cells into PRL-secreting lactotrophs. It was also reported that persistent activation of ERK/MAPK signaling not only failed to promote cell proliferation, but also reduced tumorigenesis of GH4 cells *in vitro* and *in vivo*[38]. These findings demonstrated that

ERK1/2 activation induced by OMP deficiency was not associated with tumorigenesis but promoted PRL production and secretion.

Pituitary PRL secretion is regulated by endocrine neurons in the hypothalamus. The most important of these are the neurosecretory tuberoinfundibulum neurons that secrete dopamine, which acts on lactotrophs to inhibit PRL secretion. In contrast, TRH stimulates PRL release. TRH receptor (TRHR) activation induces PKC, phosphatidylinositol, and Ca^{2+} signaling pathways[39,40]. We observed that Cab and E2 treatment similarly inhibited and stimulated PRL production in GH4 cells, respectively, irrespective of the presence or absence of OMP. However, TRH did not induce an increase in intracellular Ca^{2+} and ERK1/2 phosphorylation in cells and mice lacking OMP (Figure 5B,6B and 8A). We speculated that OMP modulated Ca^{2+}-mediated PKC/MAPK signaling, which is the main pathway responsible for TRH-induced PRL expression. Previous studies have shown that OMP modulates Ca^{2+} extrusion from olfactory sensory neurons by directly interacting with brain-expressed X-linked (Bex)1 protein, which can also bind to the Ca^{2+} dependent modulator, calmodulin (CaM)[8,10,11,41,42]. The OMP-Bex1-CaM interaction can explain the desensitization to TRH stimulation that is observed in the absence of OMP. Moreover, it has been reported that the Ca^{2+}-CaM complex itself modulates PRL gene expression. These findings indicate that OMP plays a role in PRL production and secretion in lactotrophs through the modulation of Ca^{2+} and TRH signaling.

The observed desensitization might also have been due to the downregulation of TRHR in lactotrophs in the absence of OMP, as reduction in receptor density is an important mechanism for modulating cell responsiveness[28,43]. Interestingly, TRHR expression was decreased in GH4 cells and mice lacking OMP (Figure 11). However, it is still unclear whether this effect is due to decreased transcription or increased mRNA degradation.

PRL plays a critical role in reproductive function; hyperprolactinemia is associated with anovulation and may directly or indirectly cause infertility[44]. We found that circulating PRL levels were increased in mice lacking OMP (Figure 7), which could explain the sub-fertile phenotype of these mice (https://www.jax.org/strain/006667). Interestingly, OMP was exclusively expressed in the normal pituitary gland, and not in prolactinoma tissues. Moreover, there was a negative correlation between PKC-ERK1/2 activation and low OMP expression (Figure 9). The regulation of OMP by PKC-ERK1/2 is supported by reports that OMP can modulate olfactory signaling via Ca^{2+} extrusion[5,8,10,11,45]. We proposed that the absence of OMP in lactotrophs caused abnormalities in PRL synthesis and secretion.

V. CONCLUSION

In conclusion, this study provides evidence that OMP in lactotrophs of the anterior pituitary gland plays a role in normal production and secretion of PRL, and that associated molecules function in TRH-induced PRL secretion. These findings can aid in the development of improved strategies for managing hyperprolactinemia.

REFERENCES

1. Bern HA, Nicoll CS. The comparative endocrinology of prolactin. Recent Prog Horm Res 1968;24:681-720.
2. Bernard V, Young J, Chanson P, Binart N. New insights in prolactin: pathological implications. Nat Rev Endocrinol 2015;11:265-75.
3. Grattan DR, Kokay IC. Prolactin: a pleiotropic neuroendocrine hormone. J Neuroendocrinol 2008;20:752-63.
4. Yamada M, Shibusawa N, Ishii S, Horiguchi K, Umezawa R, Hashimoto K, et al. Prolactin secretion in mice with thyrotropin-releasing hormone deficiency. Endocrinology 2006;147:2591-6.
5. Buiakova OI, Baker H, Scott JW, Farbman A, Kream R, Grillo M, et al. Olfactory marker protein (OMP) gene deletion causes altered physiological activity of olfactory sensory neurons. Proc Natl Acad Sci U S A 1996;93:9858-63.
6. Ma M, Grosmaitre X, Iwema CL, Baker H, Greer CA, Shepherd GM. Olfactory signal transduction in the mouse septal organ. J Neurosci 2003;23:317-24.
7. Margolis FL. Olfactory marker protein (OMP). Scand J Immunol Suppl 1982;9:181-99.
8. Pyrski M, Koo JH, Polumuri SK, Ruknudin AM, Margolis JW, Schulze DH, et al. Sodium/calcium exchanger expression in the mouse and rat olfactory systems. J Comp Neurol 2007;501:944-58.
9. Ivic L, Pyrski MM, Margolis JW, Richards LJ, Firestein S, Margolis FL. Adenoviral vector-mediated rescue of the OMP-null phenotype in vivo. Nat Neurosci 2000;3:1113-20.
10. Koo JH, Gill S, Pannell LK, Menco BP, Margolis JW, Margolis FL. The interaction of Bex and OMP reveals a dimer of OMP with a short half-life. J Neurochem 2004;90:102-16.
11. Kwon HJ, Koo JH, Zufall F, Leinders-Zufall T, Margolis FL. Ca extrusion by NCX is compromised in olfactory sensory neurons of OMP mice. PLoS One 2009;4:e4260.
12. Youngentob SL, Pyrski MM, Margolis FL. Adenoviral vector-mediated rescue of the OMP-null behavioral phenotype: enhancement of odorant threshold sensitivity. Behav Neurosci 2004;118:636-42.
13. Youngentob SL, Margolis FL, Youngentob LM. OMP gene deletion results in an alteration in odorant quality perception. Behav Neurosci 2001;115:626-31.

14. Flegel C, Manteniotis S, Osthold S, Hatt H, Gisselmann G. Expression profile of ectopic olfactory receptors determined by deep sequencing. PLoS One 2013;8:e55368.
15. Kang N, Kim H, Jae Y, Lee N, Ku CR, Margolis F, et al. Olfactory marker protein expression is an indicator of olfactory receptor-associated events in non-olfactory tissues. PLoS One 2015;10:e0116097.
16. Cui T, Tsolakis AV, Li SC, Cunningham JL, Lind T, Oberg K, et al. Olfactory receptor 51E1 protein as a potential novel tissue biomarker for small intestine neuroendocrine carcinomas. Eur J Endocrinol 2013;168:253-61.
17. Kang N, Bahk YY, Lee N, Jae Y, Cho YH, Ku CR, et al. Olfactory receptor Olfr544 responding to azelaic acid regulates glucagon secretion in alpha-cells of mouse pancreatic islets. Biochem Biophys Res Commun 2015;460:616-21.
18. Leja J, Essaghir A, Essand M, Wester K, Oberg K, Totterman TH, et al. Novel markers for enterochromaffin cells and gastrointestinal neuroendocrine carcinomas. Mod Pathol 2009;22:261-72.
19. Romano D, Magalon K, Ciampini A, Talet C, Enjalbert A, Gerard C. Differential involvement of the Ras and Rap1 small GTPases in vasoactive intestinal and pituitary adenylyl cyclase activating polypeptides control of the prolactin gene. J Biol Chem 2003;278:51386-94.
20. Wagle MC, Kirouac D, Klijn C, Liu B, Mahajan S, Junttila M, et al. A transcriptional MAPK Pathway Activity Score (MPAS) is a clinically relevant biomarker in multiple cancer types. NPJ Precis Oncol 2018;2:7.
21. Cakir M, Grossman AB. Targeting MAPK (Ras/ERK) and PI3K/Akt pathways in pituitary tumorigenesis. Expert Opin Ther Targets 2009;13:1121-34.
22. Arita J, Kojima Y, Kimura F. Identification by the sequential cell immunoblot assay of a subpopulation of rat dopamine-unresponsive lactotrophs. Endocrinology 1991;128:1887-94.
23. Boockfor FR, Frawley LS. Functional variations among prolactin cells from different pituitary regions. Endocrinology 1987;120:874-9.
24. Castillo AI, Tolon RM, Aranda A. Insulin-like growth factor-1 stimulates rat prolactin gene expression by a Ras, ETS and phosphatidylinositol 3-kinase dependent mechanism. Oncogene 1998;16:1981-91.
25. Schweppe RE, Frazer-Abel AA, Gutierrez-Hartmann A, Bradford AP. Functional components of fibroblast growth factor (FGF) signal transduction in pituitary cells. Identification of FGF response elements in the prolactin gene. J Biol Chem 1997;272:30852-9.

26. Wang YH, Maurer RA. A role for the mitogen-activated protein kinase in mediating the ability of thyrotropin-releasing hormone to stimulate the prolactin promoter. Mol Endocrinol 1999;13:1094-104.
27. Watters JJ, Chun TY, Kim YN, Bertics PJ, Gorski J. Estrogen modulation of prolactin gene expression requires an intact mitogen-activated protein kinase signal transduction pathway in cultured rat pituitary cells. Mol Endocrinol 2000;14:1872-81.
28. Hinkle PM, Nelson EJ, Ashworth R. Characterization of the calcium response to thyrotropin-releasing hormone in lactotrophs and GH cells. Trends Endocrinol Metab 1996;7:370-4.
29. Potter SM, Zheng C, Koos DS, Feinstein P, Fraser SE, Mombaerts P. Structure and emergence of specific olfactory glomeruli in the mouse. J Neurosci 2001;21:9713-23.
30. Kang N, Koo J. Olfactory receptors in non-chemosensory tissues. BMB Rep 2012;45:612-22.
31. Morita R, Hirohashi Y, Torigoe T, Ito-Inoda S, Takahashi A, Mariya T, et al. Olfactory Receptor Family 7 Subfamily C Member 1 Is a Novel Marker of Colon Cancer-Initiating Cells and Is a Potent Target of Immunotherapy. Clin Cancer Res 2016;22:3298-309.
32. Neuhaus EM, Zhang W, Gelis L, Deng Y, Noldus J, Hatt H. Activation of an olfactory receptor inhibits proliferation of prostate cancer cells. J Biol Chem 2009;284:16218-25.
33. Kiley SC, Parker PJ, Fabbro D, Jaken S. Differential regulation of protein kinase C isozymes by thyrotropin-releasing hormone in GH4C1 cells. J Biol Chem 1991;266:23761-8.
34. Shimon I, Huttner A, Said J, Spirina OM, Melmed S. Heparin-binding secretory transforming gene (hst) facilitates rat lactotrope cell tumorigenesis and induces prolactin gene transcription. J Clin Invest 1996;97:187-95.
35. Tashjian AH, Jr. Clonal strains of hormone-producing pituitary cells. Methods Enzymol 1979;58:527-35.
36. Banihashemi B, Albert PR. Dopamine-D2S receptor inhibition of calcium influx, adenylyl cyclase, and mitogen-activated protein kinase in pituitary cells: distinct Galpha and Gbetagamma requirements. Mol Endocrinol 2002;16:2393-404.
37. Liu JC, Baker RE, Sun C, Sundmark VC, Elsholtz HP. Activation of Go-coupled dopamine D2 receptors inhibits ERK1/ERK2 in pituitary cells. A key step in the transcriptional suppression of the prolactin gene. J Biol Chem 2002;277:35819-25.

38. Booth A, Trudeau T, Gomez C, Lucia MS, Gutierrez-Hartmann A. Persistent ERK/MAPK activation promotes lactotrope differentiation and diminishes tumorigenic phenotype. Mol Endocrinol 2014;28:1999-2011.
39. Kim GD, Carr IC, Anderson LA, Zabavnik J, Eidne KA, Milligan G. The long isoform of the rat thyrotropin-releasing hormone receptor down-regulates Gq proteins. J Biol Chem 1994;269:19933-40.
40. Sun Y, Lu X, Gershengorn MC. Thyrotropin-releasing hormone receptors similarities and differences. J Mol Endocrinol 2003;30:87-97.
41. Fernandez EM, Diaz-Ceso MD, Vilar M. Brain expressed and X-linked (Bex) proteins are intrinsically disordered proteins (IDPs) and form new signaling hubs. PLoS One 2015;10:e0117206.
42. White BA. Evidence for a role of calmodulin in the regulation of prolactin gene expression. J Biol Chem 1985;260:1213-7.
43. Gershengorn MC. Bihormonal regulation of the thyrotropin-releasing hormone receptor in mouse pituitary thyrotropic tumor cells in culture. J Clin Invest 1978;62:937-43.
44. Crosignani PG. Management of hyperprolactinemia in infertility. J Reprod Med 1999;44:1116-20.
45. Reisert J, Yau KW, Margolis FL. Olfactory marker protein modulates the cAMP kinetics of the odour-induced response in cilia of mouse olfactory receptor neurons. J Physiol 2007;585:731-40.

ABSTRACT (IN KOREAN)

후각표지단백질에 의한 뇌하수체 프로락틴분비세포의 프로락틴 분비 조절 기전 연구

< 지도교수 이은직 >

연세대학교 대학원 의과학과

강 찬 우

후각표지단백질 (OMP)은 후각 상피 내의 후각 수용세포에서 특징적으로 발현되는 세포질 단백질이다. 최근 시퀀싱 기술이 발달하면서 OMP가 내분비계와 같은 후각 기관계가 아닌 조직에서도 발현되는 것으로 알려졌다. 본 연구에서는 OMP가 인간과 마우스 뇌하수체 전엽의 프로락틴 분비세포에서 발현하는 것을 확인하였고, OMP가 후각수용세포의 세포내 칼슘을 조절하여 신호전달의 종결을 조절하는 것처럼 프로락틴 호르몬 분비에 미치는 영향 및 기전을 연구하였다. 프로락틴을 분비하는 GH4 세포에서 OMP발현을 억제하자 ERK1/2의 인산화가 증가되었고 프로락틴 생성 및 분비를 증가시켰다. 또한, Real-time PCR 분석과 칼슘 유입분석 결과를 통해 OMP가 다른 신경호르몬 도파민과 에스트로젠이 아닌 갑상샘자극 호르몬-분비호르몬에 의한 프로락틴 생성 및 분비에 관여하는 것을 확인하였다. OMP가

결핍된 마우스에서는 대조군 마우스에 비해 ERK1/2 활성 증가 및 기저 프로락틴 분비가 증가되었고, 생식능력이 떨어지는 표현형을 보였다. OMP에 의해 증가된 ERK1/2 인산화는 세포증식에는 관여하지 않고 프로락틴의 생산과 분비에만 관여하였다. 프로락틴 분비 뇌하수체선종 환자에서는 ERK1/2 인산화 및 프로락틴 발현이 정상인의 조직에 비교했을 때 확연히 증가되어 있는 반면, OMP의 발현은 정상인에 비해 확연하게 감소되어 있었다. 결론적으로, OMP는 칼슘과 갑상샘자극호르몬-분비호르몬 신호 기전을 조절하여 프로락틴 분비세포의 프로락틴 생산과 분비에 중요한 역할을 담당한다고 할 수 있다.

핵심되는 말 : 후각표지단백질, 프로락틴, 갑상샘자극호르몬분비호르몬, 프로락틴분비세포

PUBLICATION LIST

1. Kang CW, Han YE, Lee MK, Cho YH, Kang NN, Koo J, Ku CR, Lee Olfactory marker protein regulates prolactin secretion and production by modulating Ca2+ and TRH signaling in lactotrophs. Exp Mol Med. 2017;50:15-26.

2. Lee HJ, Lee WK, Kang CW, Ku CR, Cho YH, Lee EJ
(LEE011) inhibits cell proliferation and induces apoptosis in aggressive thyroid cancer. Cancer Lett. 2018;417:131-140.

3. Han YE, Kang CW, Oh JH, Park SH, Ku CR, Cho YH, Lee MK, Lee EJ Olfactory receptor OR51E1 mediates GLP-1 secretion in human and rodent enteroendocrine L cells. JES. 2018;2:1251-1258

4. Kang CW, Han YE, Kim J, Oh JH, Cho YH, Lee EJ
4-Hydroxybenzaldehyde accelerates acute wound healing through activation of focal adhesion signalling in keratinocytes. Sci Rep. 2017;7:14192-14203.

5. Byun JW, Hwang S, Kang CW, Kim JH, Chae MK, Yoon JS, Lee EJ Therapeutic Effect of Protocatechuic Aldehyde in an In Vitro Model of Graves' Orbitopathy. Invest Ophthalmol Vis Sci. 2016;15:1627-1636.