HIF-1α-Dependent Induction of Carboxypeptidase A4 and Carboxypeptidase E in Hypoxic Human Adipose-Derived Stem Cells

Yunwon Moon¹,²,³, Ramhee Moon¹,³, Hyunsoo Roh¹, Soojeong Chang¹, Seongyeol Lee¹, and Hyunsung Park¹,*

¹Department of Life Science, University of Seoul, Seoul 02504, Korea, ²Present address: Cell Therapy Research Center, GC LabCell, Yongin 16924, Korea, ³These authors contributed equally to this work.

*Correspondence: hspark@uos.ac.kr
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Hypoxia induces the expression of several genes through the activation of a master transcription factor, hypoxia-inducible factor (HIF)-1α. This study shows that hypoxia strongly induced the expression of two carboxypeptidases (CP), CPA4 and CPE, in an HIF-1α-dependent manner. The hypoxic induction of CPA4 and CPE gene was accompanied by the recruitment of HIF-1α and upregulation in the active histone modification, H3K4me3, at their promoter regions. The hypoxic responsiveness of CPA4 and CPE genes was observed in human adipocytes, human adipose-derived stem cells, and human primary fibroblasts but not mouse primary adipocyte progenitor cells. CPA4 and CPE have been identified as secreted exopeptidases that degrade and process other secreted proteins and matrix proteins. This finding suggests that hypoxia changes the microenvironment of the obese hypoxic adipose tissue by inducing the expression of not only adipokines but also peptidases such as CPA4 and CPE.

Keywords: carboxypeptidase A4, carboxypeptidase E, human adipose-derived stem cells, hypoxia, hypoxia-inducible factor-1α

INTRODUCTION

Hypoxic microenvironment has been associated with cancer progression and stemness maintenance. Hypoxia changes the expression of several target genes, but the mechanism underlying the hypoxia-mediated pleiotropic effects on metabolic status, cell cycle, differentiation, angiogenesis, cell migration, and metastasis is unclear (Moon et al., 2015). Hypoxia-inducible factor (HIF) is a master transcription factor comprising HIF-α and HIF-1β (also known as aryl hydrocarbon nuclear translocator, ARNT) subunits. HIF-α subunit is sensitive to changes in O₂ concentration because its stability and trans-activity are regulated by two different HIF-α-specific O₂ and a-ketoglutarate-dependent dioxygenases, namely, prolyl hydroxylases (PHD) and asparaginyl hydroxylase (also known as factor-inhibiting HIF, FIH). HIF-1α is hydroxylated at proline 564 and/or 402 residues by PHD using O₂. The hydroxylated proline residues of HIF-1α are recognized by an E3 ubiquitin ligase, von Hippel Lindau protein, that initiates the ubiquitin-dependent degradation of HIF-1α. FIH hydroxylates the asparagine 803 residue of HIF-1α, and prevents the binding of the coactivators CBP/p300 to the C-terminal region of HIF-1α. These two enzymes are inactivated under hypoxia, leading to the stabilization of HIF-1α and its subsequent translocation into the nucleus to form a heterodimer.
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with HIF-1β. HIF-1 heterodimer interacts with E-box-like hypoxia-responsive element to induce the expression of target genes (Gorlach et al., 2015).

Hypoxic regions in human body are often found where O2 consumption exceeds O2 supply. HIF-1α is activated at the sight of inflammation because inflammatory processes increase O2 consumption owing to an increase in oxidation reactions catalyzed by NADPH oxidases and an increase in the production of reactive oxygen species, which are inhibitors of PHD and FIH (Lee et al., 2011). O2 supply is limited in avascular regions such as the inner core of solid tumors, stem cell niche, and the obese adipose tissue. In the obese adipose tissue, hypertrophic adipocytes move further away from blood vessels and become hypoxic. Adipocytes have been re-evaluated for their endocrine functions because they secreted many cytokines and hormones such as inflammatory cytokines, leptin, and adiponectin (Makki et al., 2013). These secreted proteins from adipocytes are termed as adipokines. In comparison with lean adipocytes, obese adipocytes up-regulate the expression and secretion of many inflammatory adipokines such as interleukin-6, leptin, and plasminogen activator inhibitor-1, most of which are target genes of HIF-1α (Lee et al., 2014). Beside adipocytes, the adipose tissue is made of other cells such as endothelial cells, macrophages, and mesenchymal stem cells. In our previous microarray analyses, we identified several novel target genes that are upregulated by hypoxia in human adipose-derived stem cells (hADSCs) (Lee et al., 2017). We first found that the expression of carboxypeptidase A4 (CPA4) and carboxypeptidase E (CPE) was highly upregulated in hypoxic hADSCs (Lee et al., 2017). The hypoxic environment of hypertrophic adipocytes increases the expression of CPA4 and CPE, which function by processing other secreted proteins and matrix proteins in the adipose tissue. This finding indicates that hypoxia changes the microenvironment of the obese adipose tissue by inducing the expression of not only adipokines but also peptidases such as CPA4 and CPE from adipose-derived mesenchymal stem cells.

MATERIALS AND METHODS

Materials
Anti-HIF-1α (610958) and anti-CPE (610758) antibodies were purchased from BD Biosciences (USA). Anti-CPA4 (ab81543) and anti-14-3-3y (MAB5700) antibodies were obtained from Abcam (USA) and R&D Systems (USA), respectively. Mouse IgG (sc-2025) and anti-H3K4me3 (9751S) were obtained from Santa Cruz Biotechnology (USA) and Cell Signaling Technology (USA), respectively. Ponceau S solution and Oil Red O stain were supplied by Sigma-Aldrich (USA).

Cell culture and adipogenesis
hADSCs (catalog No. 510070, lot No. 2033) were purchased from Invitrogen (USA) and maintained as previously described (Lee et al., 2017). IMR90 human fetal lung fibroblasts were obtained from the American Type Culture Collection (ATCC, USA) and maintained in an Eagle’s minimum essential medium (EMEM) supplemented with 10% (v/v) fetal bovine serum. Cells were exposed to hypoxia through incubation in a Forma anaerobic incubator (model 1029; Thermo Fisher Scientific, USA) in an atmosphere of 5% CO2, 95% N2, and 0.5% O2. HIF-1α expression was knocked down in hADSCs using a pLKO.1 lentiviral vector (Addgene, USA) encoding either control a short-hairpin RNA (shRNA) or a shRNA sequence against human HIF-1α (5′-CCA GAG AGT ATT GTG AAG TTA-3′). For adipocyte differentiation, hADSCs were cultured in an adipogenic medium (catalog No. A1007001; Invitrogen); the medium was replaced every 3 days for 12 days according to the manufacturer’s instructions. Mouse adipocyte progenitor cells were isolated from the subvascular fraction (SVF) of the subcutaneous white adipose tissue (sWAT) of an 8-week-old C57BL/6J strain using an adipose tissue progenitor isolation kit (Miltenyi Biotec, Germany) according to the manufacturer’s instructions. The SVF was obtained after collagenase digestion of the sWAT using a previously described method (Freshney, 2000). Conditioned medium (CM) was a spent medium harvested from hADSCs cultured under normoxia (21% O2) or hypoxia (0.5% O2) for 2 days (Park et al., 2013). CM was centrifuged to remove debris and its supernatant was used.

Reverse-transcription quantitative polymerase chain reaction (RT-qPCR)
Steady-state RNA expression was measured by real-time qPCR using Power SYBR Green PCR master mix on a QuantStudio 3 real-time PCR system (Applied Biosystems, USA). The Ct value of target mRNA was normalized against that of endogenous 18S rRNA (ΔCt = Ctarget – Ct18S). C is the threshold cycle of qPCR defined by the QuantStudio 3 real-time PCR system. The relative mRNA level of a target gene is obtained by 2ΔΔCt = ΔCt-treated value – ΔCt-untreated value. Pairs of forward and reverse primer sequences for RT-qPCR were as follows: human CPA4, 5′-CAA TGA AGG GCA AGA ACG GAG C-3′ and 5′-GGT CAG GAA AGT CTG CGG CAA T-3′; human CPE, 5′-TAC TTA GTG CCC GAG AGA GAA-3′; mouse CPA4, 5′-CAG CTG CTG ATG TAT CCC TAT G-3′; human HIF-1α, 5′-CAT CAG CTA TTT GCG TGT GA-3′; human PPARα2, 5′-GCG ATT CCT TCA CTG ATA C-3′; mouse CPA4, 5′-CAG CTG CGT ATG TAT CCC CAT G-3′ and 5′-AAC GGT CCC CCA GTT CCT CCA GGA-3′; mouse CPE, 5′-TGA GAA AGA AGG CCG TCC TAA C-3′ and 5′-GCA GAT TGG CAG AAA GCA CAA-3′.

Chromatin immunoprecipitation (ChIP)
ChIP analysis was performed as previously described (Lee et al., 2017). The Ct value of target gene from the immunoprecipitated DNA sample was normalized against the Ct value of target gene in input DNA (ΔCt = Ctarget sample – Ctarget input). The percentage of input indicated the value of 100 × 2ΔΔCt. Forward and reverse primer sequence pairs used for ChIP-qPCR were as follows: human CPA4 (−1.6 kb), 5′-ACA CAT GTG GGT GCT AGT TGT TCT-3′ and 5′-AGA GTC TTT GGT AGG AGC TG-3′; human CPA4 (−0.9 kb), 5′-GGC GGT GTG GAA AGA AGC TG-3′; human CPA4 (−0.9 kb), 5′-GGC GGT GTG GAA AGA AGC TG-3′; human CPA4 (transcription start site [TSS]), 5′-GAA TGG GAG AGG CAT TGG TAG G-3′ and 5′-CAG TGG CTA AGT CGA TTA TCT T-3′; human CPE (−0.7 kb), 5′-

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TGG TGC TTT CTT ACT GGC TTC T-3′; human CPE (−0.9 kb), 5′-CAG CAC CTT ACC AAC CAT GTC T-3′ and 5′-TGG ACA CCA TGC GGG TAA TTT-3′; human CPE (TSS), 5′-GTG GGT CAT GGG TGT CTC AAT-3′ and 5′-AGG GCT GCT GGC GTT ATT-3′; human CPE (+0.9 kb), 5′-CAG CAC CCC GTC TGA ACA-3′ and 5′-CCA TCT CCG AAA GTG GAG AAA-3′.

Statistical analysis
All quantitative measurements were performed through at least two independent experiments. For comparison between two groups, P values were calculated using the paired two-tailed Student’s t-test. A value of P < 0.05 was considered statistically significant.

RESULTS
Hypoxia increases the expression of CPA4 and CPE in hADSCs
The microarray data of hypoxic hADSCs revealed the hypoxia-mediated increase in the mRNA expression levels of several carboxypeptidases (Lee et al., 2017). Among the 21 carboxypeptidases, CPA4 and CPE mRNA expression was upregulated in response to hypoxia (Fig. 1A). RT-qPCR show that the hypoxia (0.5% O2, 48 h) increased the mRNA levels of CPA4 and CPE by 13.5- and 3.9-fold, respectively (Figs. 1B and 1C).

Fig. 1. Hypoxic induction of CPA4 and CPE gene expression in hADSCs. (A) A heat map by hierarchical clustering of log2-fold changes (Log2(FC)) in the mRNA levels of the indicated isoforms of carboxypeptidases in early passage (P6, 6th passage from harvest) hADSCs exposed to normoxic (N; 20% O2) and hypoxic (H; 48 h 0.5% O2) conditions in three sets of analyses (set 1, 2, 3). Colors in the heat map represent positive (red) and negative (green) values of Log2(FC). The microarray datasets are available from GEO under listed accession numbers (GSE69495). (B and C) mRNA levels of CPA4 (B) and CPE (C) in hADSCs (P8) exposed to normoxic and hypoxic conditions. The values on y-axis indicate 2ΔΔCt × 105 and 2ΔΔCt × 107 respectively as described in Materials and Methods section. mRNA levels were normalized against 18S rRNA level. (D and E) Western blot analysis of hADSCs cultured in serum-free DMEM under normoxic or hypoxia (48 h 0.5% O2) using anti-HIF-1α, anti-CPA4 (D), anti-CPE (E), and anti-14-3-3-γ antibodies. Two representative western analyses (marked 1 and 2) of hADSC lysates were shown. Using Image J, the band intensities for CPE protein and 14-3-3-γ protein were measured. The graphs (E) indicated the relative intensities of CPE protein normalized by the intensities of 14-3-3-γ protein which were measured from six independent western blots. The hypoxic induction of CPA4 protein could not be estimated due to low band intensities in western blots. (F) Western blot analysis of the CM used to culture hADSCs. Secreted CPE protein was detected using anti-CPE antibody. Ponceau S (Pon S) staining was performed and served as loading control. Data are presented as the mean ± SE of more than two experiments. **P < 0.01; ***P < 0.001.
Anti-CPE antibody detected the hypoxic induction of CPE protein expression in hADSCs, while anti-CPA4 antibody detected the slight increase in the level of CPA4 protein in hypoxic hADSCs (Figs. 1D and 1E). Furthermore, western blot analysis using anti-CPE antibody showed that the secreted CPE protein was higher in the hypoxic CM of hADSCs than in the normoxic CM (Fig. 1F). However, we failed to detect any secreted CPA4 in CM using anti-CPA4 antibody (data not shown). These results indicate that hypoxia increases the expression of CPA4 and CPE in hADSCs.

We exposed IMR90 human fetal lung fibroblasts to hypoxia to investigate whether hypoxia induces the expression of CPA4 and CPE in other primary human cells. In comparison with IMR90 fibroblasts, hADSCs were more sensitive to hypoxia in terms of induction of CPA4 and CPE gene expression (Figs. 2A and 2B). To test whether hypoxia also induces CPA4 or CPE gene expression in mouse primary adipocyte progenitor cells, we isolated adipocyte progenitor cells from the sWAT and exposed them to hypoxia. In contrast to the observations reported for hADSCs, hypoxia induced neither CPA4 nor CPE expression in mouse adipocyte precursor cells (Figs. 2C and 2D). Further, we failed to detect upregulation in CPA4 and CPE gene expression in response to hypoxia in mouse embryo mesenchymal C3H10T1/2 cells and mouse 3T3-L1 preadipocytes (Figs. 2C and 2D). These results indicate that human and mouse adipocyte progenitor cells respond differently to hypoxia for the induction of CPA4 and CPE gene expression.

**CPA4 and CPE genes maintain their sensitivity to hypoxia in mature adipocytes**

We also tested whether hADSCs maintain their responsive-
ness to hypoxia for the induction of CPA4 and CPE gene expression after their differentiation into mature adipocytes. We treated hADSCs with an adipogenic medium for 12 days to induce adipogenesis (Fig. 3A). Oil Red O staining and RT-qPCR analysis revealed the accumulation of lipid droplets and induction of peroxisome proliferator-activated receptor (PPAR)γ-2, a master adipogenic transcription factor, respectively, confirming the successful differentiation of hADSCs into mature adipocytes (Figs. 3B and 3C). During adipogenesis, CPA4 mRNA expression was reduced but CPE mRNA level increased (Fig. 3C). After adipogenesis, mature adipocytes were exposed to hypoxia and their mRNA levels of CPA4 and

Fig. 3. Hypoxic induction of CPA and CPE genes in human mature adipocytes. (A) Schematic diagram showing the adipogenesis process and hypoxic treatment. (B) Lipid droplets in differentiated hADSCs were visualized by Oil Red O staining (×400). For in vitro adipogenesis, hADSCs were cultured in an adipogenic medium for 12 days under normoxia (N12) then exposed to either hypoxia (0.5% O2) (N12H2) or normoxia (N14) (20% O2) for additional 2 days. (C) Relative mRNA levels of PPARγ2, CPA4, and CPE in undifferentiated hADSCs (0) and human mature adipocytes (N12). (D and E) mRNA levels of CPA4 and CPE in undifferentiated hADSCs (adipogenesis negative, −) exposed to normoxia (N) and hypoxia (H) for 2 days. Mature adipocytes (adipogenesis positive, +) were exposed to either hypoxia (N12H2) or normoxia (N14) for additional 2 days. mRNA levels were normalized against 18S rRNA level. (F) Western blot analyses of hADSCs and mature adipocytes exposed to normoxia or hypoxia (48 h 0.5% O2) using anti-HIF-1α, anti-CPE, and anti-14-3-3-γ antibodies. The representative result among two independent experiments was shown. Data are presented as the mean ± SE of more than two experiments. ***P < 0.001.
CPE were measured (Fig. 3A). Hypoxia also increased mRNA of CPA4 and CPE even in mature human adipocytes (Figs. 3D and 3E). Western blot analyses detected the hypoxic increase of CPE protein in human mature adipocytes which were differentiated from hADSC (Fig. 3F). Thus, CPA4 and CPE genes maintained their sensitivity to hypoxia after the differentiation of hADSCs into mature adipocytes.

**HIF-1α mediates hypoxia-induced CPA4 and CPE expression**

To test whether HIF-1α is essential for mediating the hypoxia-induced expression of CPA4 and CPE genes, we knocked down the expression of HIF-1α in hADSCs by infecting them with a lentivirus encoding shRNA against HIF-1α. As control, hADSCs were infected with a lentivirus encoding a control shRNA. The knockdown of HIF-1α expression resulted in a decrease in the mRNA level of HIF-1α along with a decrease in the expression of its target gene, MT3 (Figs. 4A and 4B). In control hADSCs, hypoxia induced CPA4 and CPE gene expression by 14- and 3.5-fold, respectively. In HIF-1α knockdown hADSCs, hypoxia increased the mRNA levels of CPA4 and CPE only by 1.6-fold (Figs. 4C and 4D). These results indicate that HIF-1α is essential for mediating the hypoxia-induced upregulation in both CPA4 and CPE gene expression. Using JASPAR program, we found putative HIF-1α binding sites in the upstream regions of these two genes (Figs. 4E and 4F).
ChIP-qPCR analysis showed that HIF-1α binding was detected on the promoters of CPA4, CPE, and MT3 genes (Figs. 4G-4I). These results suggest that HIF-1α binds to the promoters of both CPA4 and CPE genes and is essential for the hypoxic induction of these carboxypeptidase expression. To investigate the epigenetic changes in histone modification, we detected the hypoxia-mediated changes in a positive histone mark, H3K4me3, at the promoters of CPA4 and CPE genes. ChIP-qPCR analysis showed that H3K4me3 level significantly increased at the promoters of both CPA4 and CPE genes (Figs. 4J and 4K). Thus, hypoxia increased the transcription of CPA4 and CPE genes and this effect was accompanied by the recruitment of HIF-1α and increment in the active histone modification H3K4me3 at their promoter regions.

DISCUSSION

This study shows that hypoxic condition dramatically induced the expression of two carboxypeptidases CPA4 and CPE in an HIF-1α-dependent manner. The hypoxic responsiveness of CPA4 and CPE genes is restricted to human mesenchymal cells, and the adipocytes differentiated from these stem cells. In mouse adipocyte precursor cells, hypoxia failed to induce the expression of Cpa4 and Cpe genes (Figs. 2C and 2D). Although mice have been used for an animal model of human obesity studies, there is a lack of studies showing the similarities and differences in function and gene expression of adipose tissues between human and mouse (Chusyd et al., 2016; Lindgren et al., 2009; Vohl et al., 2004). A few comparative analyses of differentially expressed gene between WAT and brown adipose tissue revealed that human and mouse share less than ten percent of genes which are commonly regulated during browning process of adipose tissues (Baboota et al., 2015). Hypoxia increased expression of CPA4 and CPE genes in hADSCs of human but not Cpa4 and Cpe genes in predisocytes of mouse. It remains to investigate the different hypoxic signaling pathways to regulate the expressions of these genes between human and mouse. Carboxypeptidases cleave peptides from their C-termini, usually one residue at a time. CPE and CPA4 belong to the metallo-carboxypeptidase M14 family, which comprises 25 distinct genes (Sapio and Fricker, 2014). Carboxypeptidases have broad substrate specificity; the members of the A family cleave C-terminal aromatic/aliphatic residues, while those of the E subfamily remove C-terminal lysine and arginine residues without further hydrolyzing the peptide (Fricker and Snyder, 1983). CPA4 was also found to be upregulated in several tumors such as colorectal cancer, hepatocellular carcinoma, gastric cancer, esophageal squamous cell carcinoma, pancreatic cancer, and breast cancer (Handa et al., 2019; Pan et al., 2019; Sun et al., 2016a; 2016b; 2017; Zhang et al., 2019). CPA4 is closely associated with cancer metastasis. Patients with high levels of CPA4 had poorer prognosis. Serum levels of CPA4 may serve as a marker of poor prognosis and CPA4 could be a promising therapeutic target in several tumors with aggressive phenotypes. Similar to CPA4, CPE expression is detected in tissues that secrete bioactive peptides and is associated with peptide-containing secretory granules (Ji et al., 2017). CPE knockout mice show characteristics such as obesity, low bone mineral density, infertility, and neurodegeneration (Che et al., 2001; Fricker et al., 2000). Peptidomic analysis using brains of CPE knockout mice identified several CPE substrate neuropeptides, and their expression was severely dysregulated in mutant mice (Fricker et al., 2000). CPE is involved in the peptide secretion process. A membrane-associated form of CPE binds to proopiomelanocortin, a multivalent prohormone, to facilitate its trafficking from the trans-Golgi network into secretory granules (Cawley et al., 2016).

Considering that hypoxic regions are often found in the obese adipose tissue, our findings suggest the possibility that hypoxia-mediated induction of CPA4 and CPE expression in the obese adipose tissue may contribute to the upregulation in secreted CPA4 and CPE proteins in the serum, ultimately leading to increased tumor metastasis. Hypoxia is an important environmental condition that determines cell fate by reconstituting microenvironment through induction of target secretory proteins. This study suggests that hypoxic induction of secretory CPE and CPA4 proteins in hADSCs further changes the extracellular microenvironment by processing diverse extracellular substrates of CPA4 and CPE.

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AUTHOR CONTRIBUTIONS

Y.M., R.M., and H.P. conceived ideas, performed experiments and wrote the manuscript. H.R., S.C., and S.L. performed experiments.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

ORCID

Yunwon Moon https://orcid.org/0000-0003-2878-2887
Ramhee Moon https://orcid.org/0000-0001-6213-9962
Hyunsoo Roh https://orcid.org/0000-0003-4065-5186
Sojeong Chang https://orcid.org/0000-0002-1961-6687
Seongyeol Lee https://orcid.org/0000-0003-2086-9662
Hyunsung Park https://orcid.org/0000-0002-6890-428X

REFERENCES

Baboota, R.K., Sarma, S.M., Boparai, R.K., Kondepudi, K.K., Mantri, S., and Bishnoi, M. (2015). Microarray based gene expression analysis of murine brown and subcutaneous adipose tissue: significance with human. PLoS One 10, e0127701.
Cawley, N.X., Rathod, T., Young, S., Lou, H., Birch, N., and Loh, V.P. (2016). Carboxypeptidase E and secretogranin III coordinately facilitate efficient sorting of proopiomelanocortin to the regulated secretory pathway in AtT20 cells. Mol. Endocrinol. 30, 37-47.
Che, F.Y., Yan, L., Li, H., Mzhavia, N., Devi, L.A., and Fricker, L.D. (2001). Identification of peptides from brain and pituitary of Cpe<sup>−/−</sup>/Cpe<sup>+/+</sup> mice. Proc. Natl. Acad. Sci. U. S. A. 98, 9971-9976.
Chusyd, D.E., Wang, D., Huffman, D.M., and Nagy, T.R. (2016). Relationships between rodent white adipose fat pads and human white adipose fat depots. Front. Nutr. 3, 10.
Freshney, R.I. (2000). Culture of Animal Cells: A Manual of Basic Technique
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(4th Edition) (New York: Wiley).

Fricker, L.D., McKinzie, A.A., Sun, J., Curran, E., Qian, Y., Yan, L., Patterson, S.D., Courchesne, P.L., Richards, B., Levin, N., et al. (2000). Identification and characterization of proSAAS, a granin-like neuroendocrine peptide precursor that inhibits prohormone processing. J. Neurosci. 20, 639-648.

Fricker, L.D. and Snyder, S.H. (1983). Purification and characterization of enkephalin convertase, an enkephalin-synthesizing carboxypeptidase. J. Biol. Chem. 258, 10950-10955.

Gorlach, A., Dimova, E.Y., Petry, A., Martinez-Ruiz, A., Hernansanz-Agustin, P., Rolo, A.P., Palmeira, C.M., and Kietzmann, T. (2015). Reactive oxygen species, nutrition, hypoxia and diseases: problems solved? Redox Biol. 6, 372-385.

Handa, T., Katayama, A., Yokobori, T., Yamane, A., Fujii, T., Obayashi, S., Kurozumi, S., Kawabata-Iwakawa, R., Gombodorj, N., Nishiyama, M., et al. (2019). Carboxypeptidase A4 accumulation is associated with an aggressive phenotype and poor prognosis in triple-negative breast cancer. Int. J. Oncol. 54, 833-844.

Ji, L., Wu, H.T., Qiu, X.Y., and Lan, R. (2017). Dissecting carboxypeptidase E: properties, functions and pathophysiological roles in disease. Endocr. Connect. 6, R18-R38.

Lee, H.Y., Lee, T., Lee, N., Yang, E.G., Lee, C., Lee, J., Moon, E.Y., Ha, J., and Park, H. (2011). Src activates HIF-1α not through direct phosphorylation of HIF-1α-specific prolyl-4 hydroxylase 2 but through activation of the NADPH oxidase/Rac pathway. Carcinogenesis 32, 703-712.

Lee, S., Lee, J., Chae, S., Moon, Y., Lee, H.Y., Park, B., Yang, E.G., Hwang, D., and Park, H. (2017). Multi-dimensional histone methylations for coordinated regulation of gene expression under hypoxia. Nucleic Acids Res. 45, 11643-11657.

Lee, Y.S., Kim, J.W., Osborne, O., Oh, D.Y., Sasik, R., Schenk, S., Chen, A., Chung, H., Murphy, A., Watkins, S.M., et al. (2014). Increased adipocyte O2 consumption triggers HIF-1α, causing inflammation and insulin resistance in obesity. Cell 157, 1399-1352.

Lindgren, C.M., Heid, I.M., Randall, J.C., Lamina, C., Steinhorsdottir, V., Qi, L., Speliotes, E.K., Thorleifsson, G., Willer, C.J., Herna, B.M., et al. (2009). Genome-wide association scan meta-analysis identifies three Loci influencing adiposity and fat distribution. PLoS Genet. 5, e1000508.

Makki, K., Frogluc, P., and Wolowczuk, I. (2013). Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. ISRN Inflamm. 2013, 139239.

Moon, Y., Choi, S.M., Chang, S., Park, B., Lee, S., Lee, M.O., Choi, H.S., and Park, H. (2015). Chenodeoxycholic acid reduces hypoicducible factor-1α protein and its target genes. PLoS One 10, e0130911.

Pan, H., Pan, J., Ji, L., Song, S., Lu, H., Yang, Z., and Guo, Y. (2019). Carboxypeptidase A4 promotes cell growth via activating STAT3 and ERK signaling pathways and predicts a poor prognosis in colorectal cancer. Int. J. Biol. Macromol. 138, 125-134.

Park, Y.K., Park, B., Lee, S., Choi, K., Moon, Y., and Park, H. (2013). Hypoxia-inducible factor-2α-dependent hypoicducible induction of Wnt10b expression in adipogenic cells. J. Biol. Chem. 288, 26311-26322.

Sapio, M.R. and Fricker, L.D. (2014). Carboxypeptidases in disease: insights from peptidomic studies. Proteomics Clin. Appl. 8, 327-337.

Sun, L., Burnett, J., Guo, C., Xie, Y., Pan, J., Yang, Z., Ran, Y., and Sun, D. (2016a). CPA4 is a promising diagnostic serum biomarker for pancreatic cancer. Am. J. Cancer Res. 6, 91-96.

Sun, L., Cao, J., Guo, C., Burnett, J., Yang, Z., Ran, Y., and Sun, D. (2017). Associations of carboxypeptidase 4 with ALDH1A1 expression and their prognostic value in esophageal squamous cell carcinoma. Dis. Esophagus 30, 1-5.

Sun, L., Guo, C., Yuan, H., Burnett, J., Pan, J., Yang, Z., Ran, Y., Myers, I., and Sun, D. (2016b). Overexpression of carboxypeptidase A4 (CPA4) is associated with poor prognosis in patients with gastric cancer. Am. J. Transl. Res. 8, 5071-5075.

Vohl, M.C., Sladek, R., Robitaille, J., Gurd, S., Marceau, P., Richard, D., Hudson, T.J., and Tcheren, A. (2004). A survey of genes differentially expressed in subcutaneous and visceral adipose tissue in men. Obes. Res. 12, 1217-1222.

Zhang, H., Hao, C., Wang, H., Shang, H., and Li, Z. (2019). Carboxypeptidase A4 promotes proliferation and stem cell characteristics of hepatocellular carcinoma. J. Exp. Pathol. 100, 133-138.