Molecules and Cells

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

Yunwon Moon[1,2,3], Ramhee Moon[1,3], Hyunsoo Roh[1], Soojeong Chang[1], Seongyeol Lee[1], and Hyunsung Park[1,*]

[1]Department of Life Science, University of Seoul, Seoul 02504, Korea, [2]Present address: Cell Therapy Research Center, GC LabCell, Yongin 16924, Korea, [3]These 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 O_2 concentration because its stability and trans-activity are regulated by two different HIF-α-specific O_2 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 O_2. 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 O_2 consumption exceeds O_2 supply. HIF-1α is activated at the sight of inflammation because inflammatory processes increase O_2 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). O_2 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 upregulate 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-14-3-3γ (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% CO_2, 95% N_2, and 0.5% O_2. 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 GTT ATG 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% O_2) or hypoxia (0.5% O_2) 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 C_t value of target mRNA was normalized against that of endogenous 18S rRNA ($\Delta Ct = C_{t\ target} - C_{t\ 18S}$). C_t 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 $2^{-\Delta\Delta Ct}$; $\Delta\Delta C_t = \Delta C_{t\ treated\ value} - \Delta C_{t\ untreated\ value}$. 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′-CTT GGC CCA GTA CCT ATG CAA-3′ and 5′-ACC AGT CCT TGA GTT CAC CAG-3′; human *PPARγ2*, 5′-GCG ATT CCT TCA CTG ATA C-3′ and 5′-CTT CCA TTA CGG AGA GAT CC-3′; human *HIF-1α*, 5′-CAT CAG CTA TTT GCG TGT GAG GA-3′ and 5′-AGC AAT TCA TCT GTG CTT TCA TGT C-3′; mouse *CPA4*, 5′-CAG CTG CTG ATG TAT CCC TAT G-3′ and 5′-TAC TTA GTG CCC GAG AGA GAA-3′; mouse *CPE*, 5′-TGA GAA AGA AGG CGG 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 C_t value of target gene from the immunoprecipitated DNA sample was normalized against the C_t value of target gene in input DNA ($\Delta C_t = C_t^{sample} - C_t^{input}$). The percentage of input indicated the value of $100 \times 2^{\Delta Ct}$. Forward and reverse primer sequence pairs used for ChIP-qPCR were as follows: human *CPA4* (–1.6 kb), 5′-ACA GAT TTG GGT CTC AGT TGT TCT-3′ and 5′-AGA GCC TTT GTT AGG AGC TGT G-3′; human *CPA4* (–0.9 kb), 5′-GGC GTT ATG GAG ATA TGA GTG TCT TC-3′ and 5′-CTT CCT AGA CTG TGG GTC TGG T-3′; human *CPA4* (transcription start site [TSS]), 5′-GAA TGG GAG AGG CAT TGG TAG G-3′ and 5′-CAG TGG CTG AGT CAA GCT GTA T-3′; human *CPA4* (+0.7 kb), 5′-

TGG TGC TTT CTT ACT GGC TTC T-3′ and 5′-CCT ACC ACC CAA ATG TCC TCT C-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′-ACC TTA GGC CAC CCT CTA GTA A-3′ and 5′-ACT CTT GTC AGC AAG GAA GAC C-3′; human *MT3* (–0.4 kb), 5′-CAG CAA 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% O_2, 48 h) increased the mRNA levels of *CPA4* and *CPE* by 13.5- and 3.9-fold, respectively (Figs. 1B and

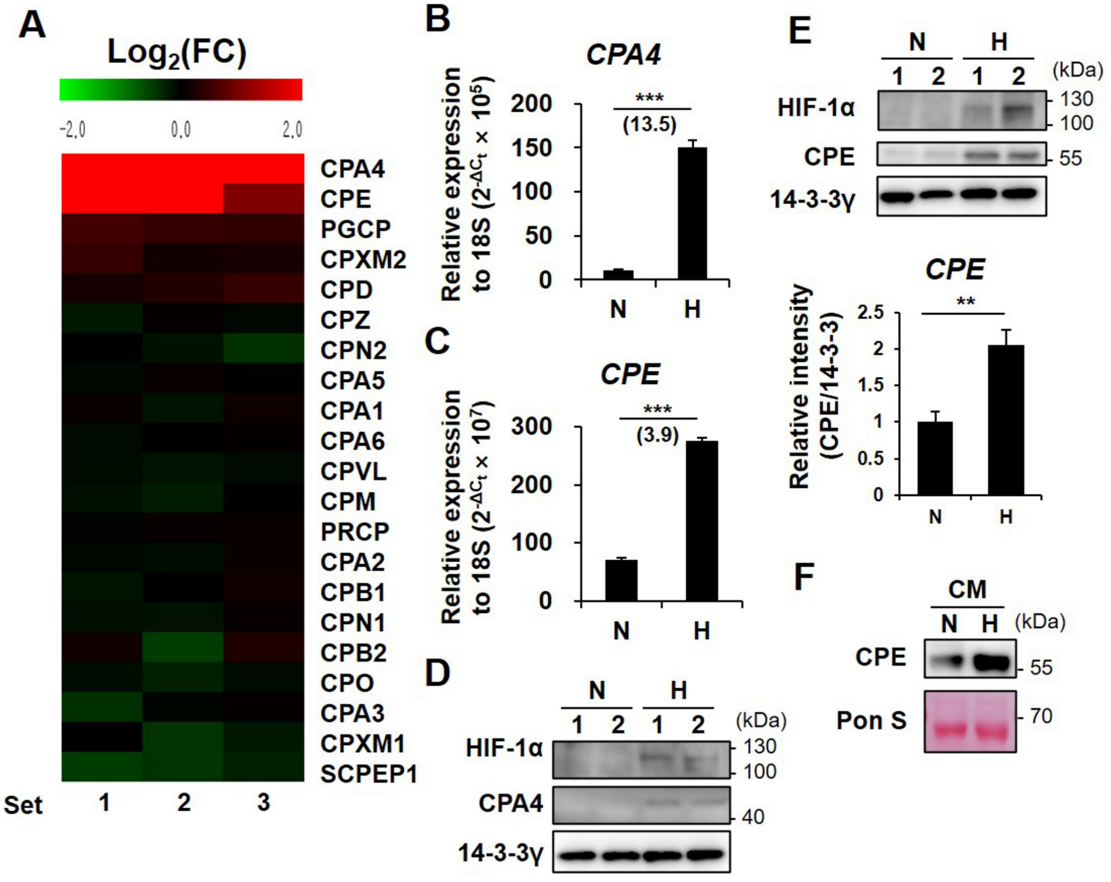

Fig. 1. Hypoxic induction of *CPA4* and *CPE* gene expression in hADSCs. (A) A heat map by hierarchical clustering of $\log_2$-fold changes (Log_2(FC)) in the mRNA levels of the indicated isoforms of carboxypeptidases in early passage (P6, 6th passage from harvest) hADSCs exposed to normoxic (N; 20% O_2) and hypoxic (H; 48 h 0.5% O_2) conditions in three sets of analyses (set 1, 2, 3). Colors in the heat map represent positive (red) and negative (green) values of Log_2(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 $2^{-\Delta Ct} \times 10^5$ and $2^{-\Delta Ct} \times 10^7$ 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 normoxia or hypoxia (48 h 0.5% O_2) using anti-HIF-1α, anti-CPA4 (D), anti-CPE (E), and anti-14-3-3γ antibodies. Two representative western analyses (marked 1 and 2) of hADSC lysates were shown. Using Image J, the band intensities for CPE protein and 14-3-3γ protein were measured. The graphs (E) indicated the relative intensities of CPE protein normalized by the intensities of 14-3-3γ 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. $^{**}P < 0.01$; $^{***}P < 0.001$.

1C). 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-

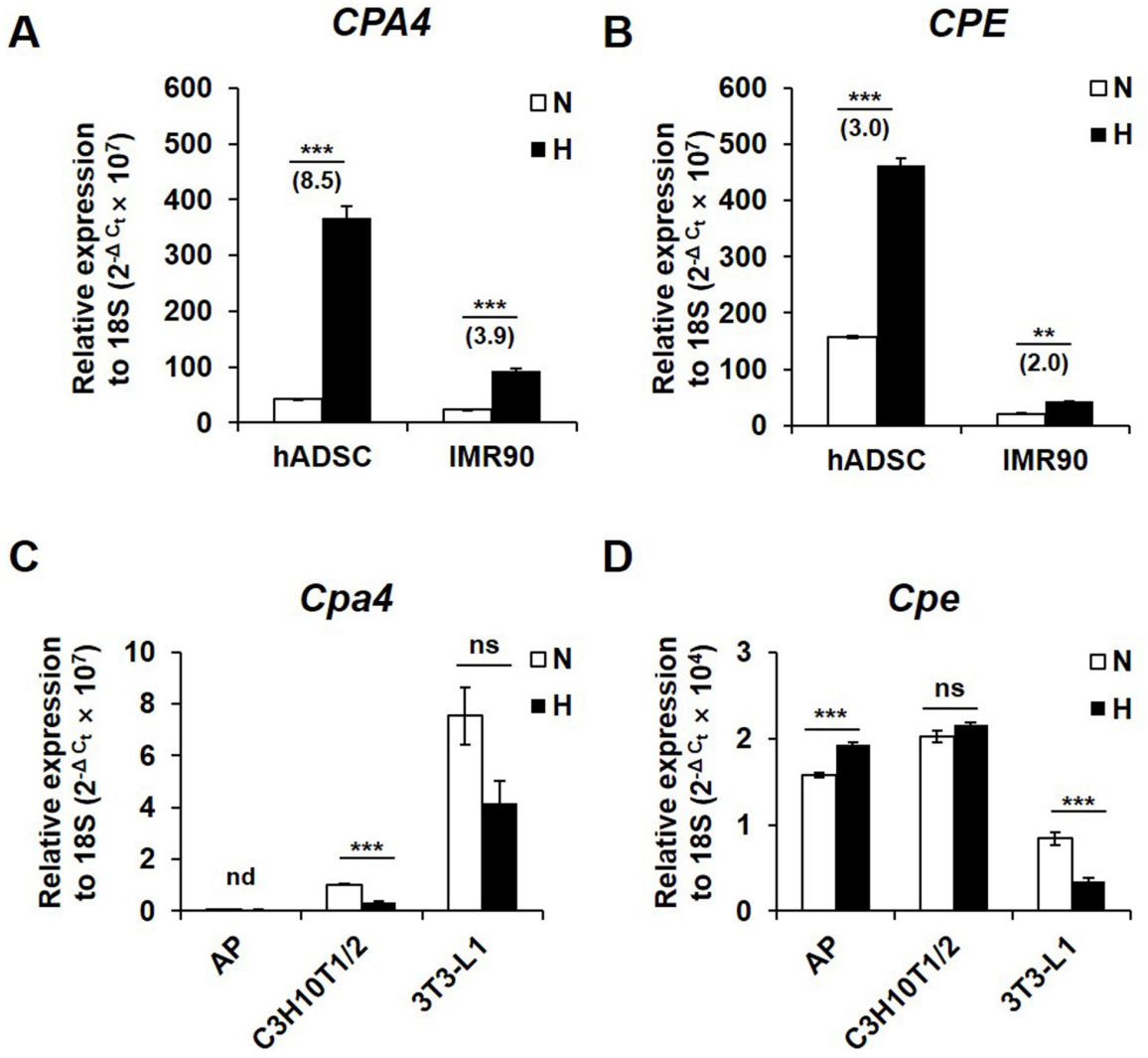

Fig. 2. Hypoxic induction of *CPA4* and *CPE* genes in other cell lines. (A and B) mRNA levels of *CPA4* (A) and *CPE* (B) in hADSCs (P8) and IMR90 human fetal lung fibroblasts. The values on y-axis indicates $2^{-\Delta Ct} \times 10^7$ as described in Materials and Methods section. mRNA levels were normalized against 18S rRNA level. (C and D) mRNA levels of mouse *Cpa4* (C) and *Cpe* (D) in normoxic (N) and hypoxic (H) mouse primary adipocyte progenitor (AP) cells, C3H10T1/2 mouse pluripotent mesenchymal cells, and 3T3-L1 mouse preadipocytes. Data are presented as the mean ± SE of more than two experiments. $^{**}P < 0.01$; $^{***}P < 0.001$; nd, not detected; ns, not significant $P > 0.05$.

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

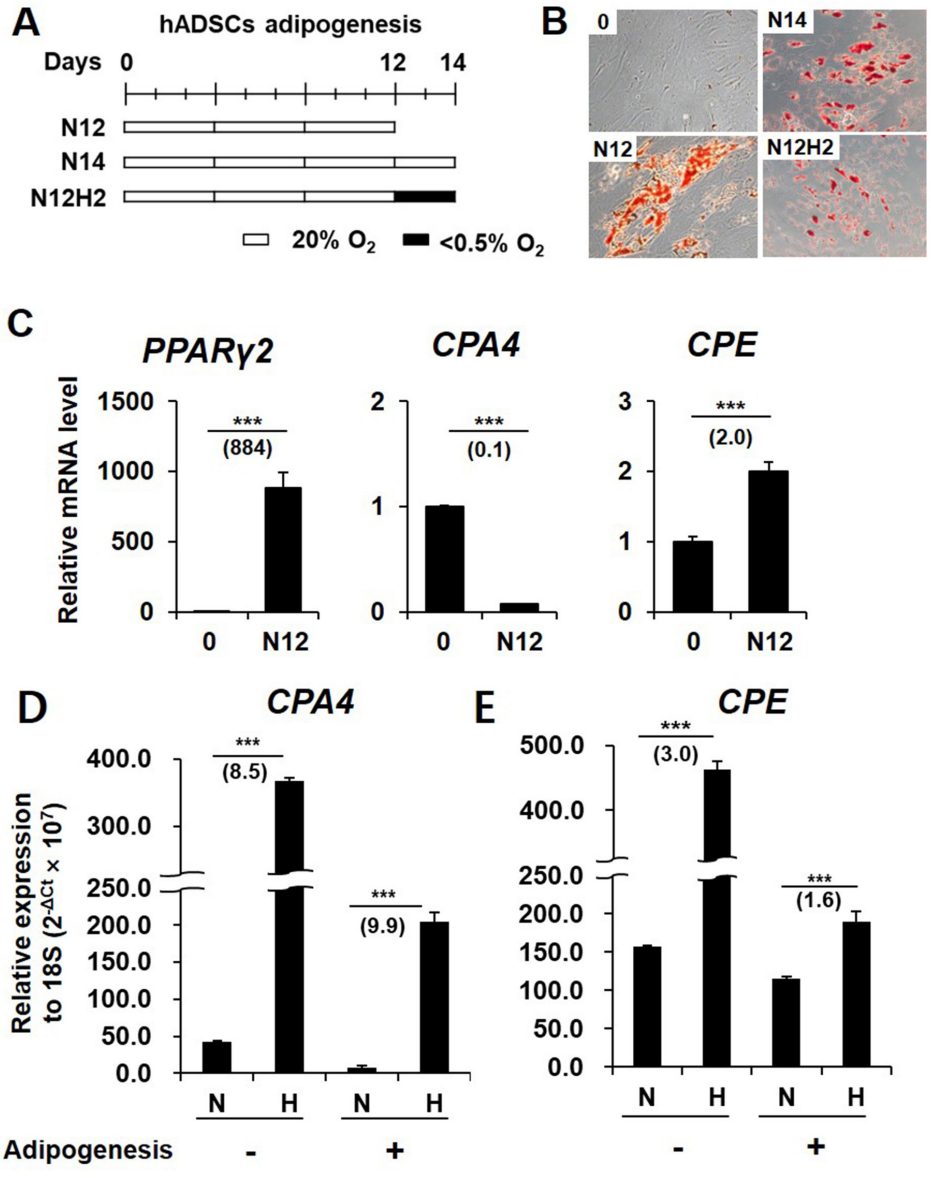

Fig. 3. Hypoxic induction of *CPA* and *CPE* genes in human mature adipocytes. (A) Schematic diagram showing the adipogenesis process and hypoxic treatment. (B) Lipid droplets in differentiated hADSCs were visualized by Oil Red O staining (×400). For *in vitro* adipogenesis, hADSCs were cultured in an adipogenic medium for 12 days under normoxia (N12) then exposed to either hypoxia (0.5% O_2) (N12H2) or normoxia (N14) (20% O_2) 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% O_2) using anti-HIF-1α, anti-CPE, and anti-14-3-3γ 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).

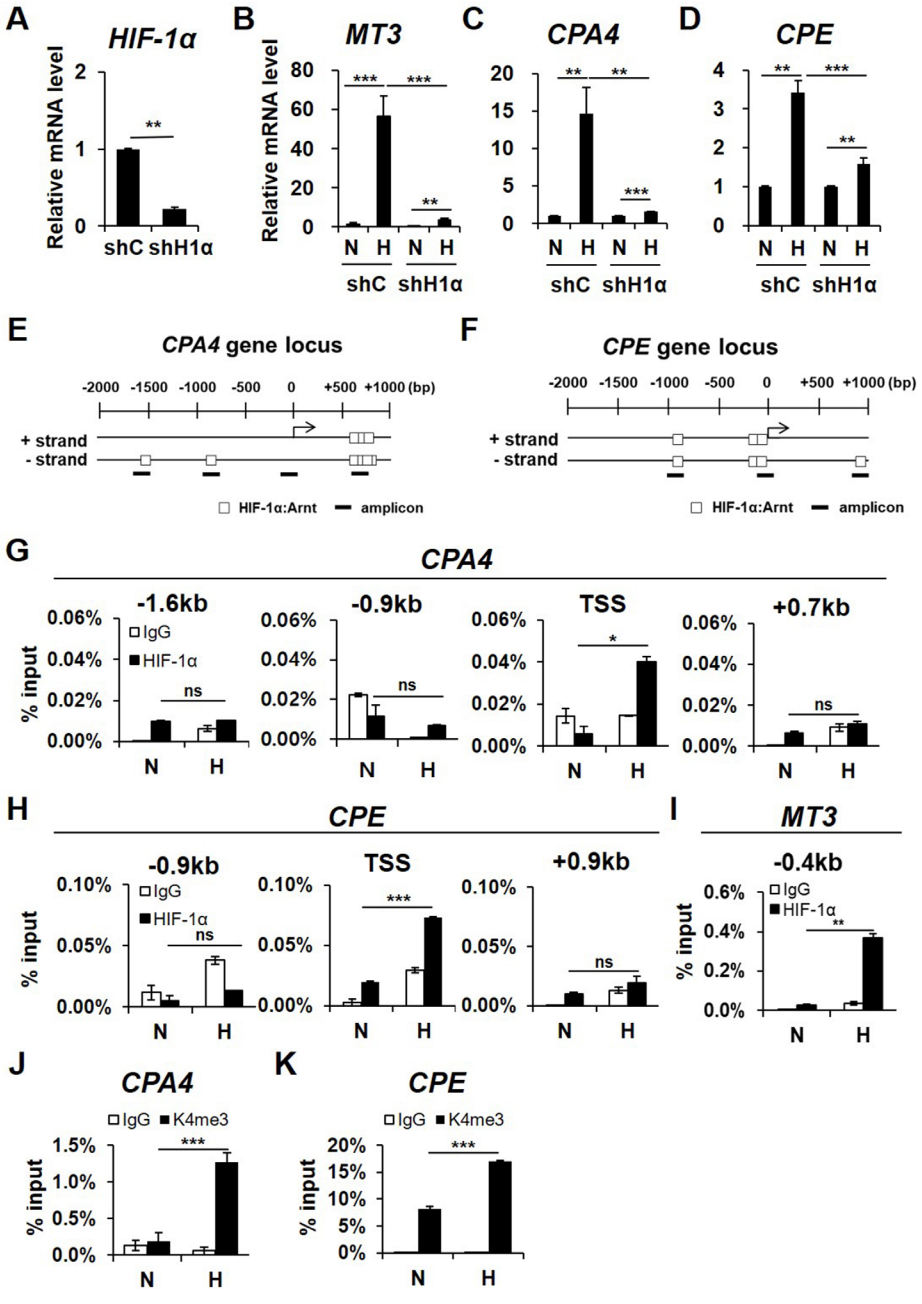

Fig. 4. HIF-1α-dependent induction of *CPA* and *CPE* gene expression in hypoxic hADSCs. (A) Relative mRNA levels of *HIF-1α* in HIF-1α-knockdown hADSCs (shH1α) and control hADSCs (shC). (B-D) Relative mRNA levels of *MT3* (B), *CPA4* (C), and *CPE* (D) in either shC or shH1α hADSCs cultured under normoxia (N) or hypoxia (H; 48 h, 0.5% O_2). (E and F) Diagrams of putative HIF-1α/ARNT-binding sites on human *CPA4* (E) and *CPE* (F) genes from -2 kb to 1 kb from the TSS. The HIF-1α/ARNT-binding sites with relative scores above 0.8 in JASPAR analyses are shown. Black bars indicate the amplicon targeted by ChIP-qPCR primer sets for the upstream region, TSS, and gene body of *CPA4* and *CPE* genes. (G-I) ChIP-qPCR analyses of HIF-1α occupancy on the indicated regions of *CPA4* (G) and *CPE* (H) and -0.4 kb from the TSS of *MT3* gene (I) in hADSCs under normoxia or hypoxia (48 h 0.5% O_2). The values on y-axis indicate the percentage of input as described in Materials and Methods section. (J and K) ChIP-qPCR analyses of H3K4me3 at the TSS of *CPA4* (J) and *CPE* (K). Data are presented as the mean ± SE of more than two experiments. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; ns, not significant $P > 0.05$.

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 hADSC of human but not *Cpa4* and *Cpe* genes in preadipocytes 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 CPE and CPA4.

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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
Soojeong 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

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