Hypoxia inducible factor-1α regulates autophagy via the p27-E2F1 signaling pathway

PAN WANG[1,2], MEIJING LONG[1,2], SHIJIE ZHANG[1,2], ZHENYUN CHENG[1,2], XIN ZHAO[1,2], FUCHENG HE[1,2], HONGCHUN LIU[1,2] and LIANG MING[1,2]

[1]Department of Clinical Laboratory; [2]Key Clinical Laboratory of Henan, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, P.R. China

Received August 20, 2016; Accepted June 1, 2017

DOI: 10.3892/mmr.2017.6794

Abstract. Autophagy is a highly conserved process by which the cell contents are delivered to lysosomes for degradation, or are used to provide macromolecules for energy generation under conditions of nutritional starvation. It has previously been demonstrated that cancer cells in hypoxic regions, with an oxygen concentration below the normal physiological level, express hypoxia inducible factor (HIF)-1α, in order to adapt and survive. HIF-1α is important in the regulation of oxygen homeostasis and the transcription of hundreds of genes in response to conditions of hypoxia, hence maintaining energy and redox homeostasis. To determine if HIF-1α modulates autophagy and the underlying molecular mechanisms regulating this process, the human esophageal cancer EC109 and IMR90 human diploid fibroblast cell lines were exposed to normoxic or hypoxic conditions and the expression levels of various proteins subsequently examined. Small interfering RNA was used to silence p27, in order to investigate its role in the process of HIF-1α regulated autophagy. Hypoxia induced autophagy in IMR90 cells and it was revealed that immature IMR90 cells demonstrated an increased rate of autophagy compared with mature cells. HIF-1α promoted EC109 cell autophagy via positively modulating p27, whereas silencing of p27 abolished the autophagy induced by hypoxia. The present study identified the primary components of the p27-E2F1 signaling pathway by which HIF-1α regulates autophagy. A previously unidentified mechanism is here presented, via which cancer cells may generate energy, or obtain macromolecules for survival.

Correspondence to: Dr Shijie Zhang or Dr Liang Ming, Department of Clinical Laboratory, The First Affiliated Hospital of Zhengzhou University, 1 Jianshe E Road, Zhengzhou, Henan 450052, P.R. China
E-mail: zsjsuccess@126.com
E-mail: mingliang3072@163.com

Key words: HIF-1α, p27, E2F1, LC-3, autophagy

Introduction

Autophagy is a highly conserved, physiological, catabolic process that engulfs organelles and cytoplasmic contents, including macromolecules such as proteins and lipids (1,2). These are broken down to their basic components to sustain cellular metabolism. In addition to providing a basic catabolic function, autophagy is believed to be essential for the maintenance of cellular homeostasis via coping with stressful conditions to improve survival (3). Unlike the ubiquitin-proteasome system which selectively degrades proteins attached by ubiquitin (4), autophagy nonselectively degrades cytoplasmic proteins and dysfunctional organelles (5). In mammalian cells, there are predominantly three autophagic pathways that have been identified, including macroautophagy, microautophagy and chaperone-mediated autophagy (6,7).

Emerging evidence suggests that autophagy plays a context-dependent role in cancer (8-10), autophagy suppresses tissue injury and tumor initiation by elimination of damaged cellular components on one side, however, in an established tumor, autophagy promotes cancer progression by providing substrates for metabolism and fostering survival (11,12). The survival of organisms is dependent upon their ability to efficiently generate energy through the process of mitochondrial oxidative phosphorylation and when cells subjected to prolonged hypoxia, autophagy is an adaptive metabolic response to let cells go through energy deficiency and this process requires the hypoxia-inducible factor 1 to maintain oxygen homestasis (13-15).

HIF-1 composed of a constitutively expressed HIF-1β subunit and an O_2-regulated HIF-1α subunit is a heterodimer and plays a key role in the regulation of oxygen homestasis (16,17). Under aerobic conditions, HIF-1α subunit is rapidly degraded but stabilized when the O_2 dependent prolyl hydroxylases (PHDs) are inhibited under hypoxia (16). HIF-1 regulates the transcription of hundreds of genes in response to hypoxia whose products restore blood supply and nutrients (18,19). So far, there is growing evidence suggested autophagy is linked to hypoxia, however an understanding of the precise role of HIF-1 in the course of autophagy remains dismal.

In this present study, we examined the role of HIF-1α/p27 in the regulation of autophagy. Our data indicate that HIF-1α

induces autophagy by promoting p27 activity, and p27 silence inhibits HIF-1α induced autophagy. HIF-1α could also promote esophageal carcinoma cells proliferation and tumorigenesis in xenograft.

Materials and methods

Cell lines, cell culture. Human esophageal cancer EC109 and IMR90 human diploid fibroblasts cells were purchased from National Institute of Biological Products, Beijing, China. Young IMR90 cells are defined as having completed <30 PD, while replicative senescent IMR90 cells are defined as having completed >50 PD. EC109 cell and IMR90 cell were cultured in RPMI-1640 and DMEM media, respectively, supplemented with 10% fetal bovine serum at 37˚C. In the experiments 2.4 g/l HEPES was added into the medium to inhibit cell apoptosis caused by acidosis under hypoxia. Cultures at 90% confluence were digested with 0.25% trypsin after washing with a PBS solution and then split at a ratio of 1:2. For hypoxia culture, cells were placed in a hypoxic (1% O_2, 5% CO_2, 94% N_2, 37˚C) incubator (New Brunswick Scientific Co., Ltd., Enfield, CT, USA) for indicated time. Control cells were incubated for equivalent periods under normoxic conditions (21% O_2, 5% CO_2, 37˚C).

Plasmids, antibodies and regent. HIF-1α wild type (HIF-1α WT) plasmid and HIF-1α constitutively active form of HIF-1α (HIF-1α ΔCA) plasmid were kindly gift from Dr Makio Hayakawa. As HIF-1α can be degraded through the ubiquitin-proteasome pathway upon normoxia by von Hippel-Lindau (VHL) protein and VHL protein binds to HIF-1α by recognizing two highly conserved proline residues (Pro-402 and Pro-564) for polyubitylation, so let alanine to substitute the conserved proline will keep HIF-1α constitutively active. Transfection into EC109 cells was performed using Lipofectamine Plus (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The antibodies used in this study were antibodies against HIF-1α (BD Transduction Laboratories, Lexington, KY, USA), p16 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), p27 (Santa Cruz Biotechnology, Inc.), PTEN (Santa Cruz Biotechnology), E2F1 (Cell Signaling Technology, Inc., Danvers, MA, USA), LC3 (Cell Signaling Technology, Inc.), Bcl-2 (Cell Signaling Technology, Inc.), PARP (Santa Cruz Biotechnology, Inc.), actin (Santa Cruz Biotechnology, Inc.). Besides, $CoCl_2$ (Sigma, St. Louis, MO, USA) was dissolved in sterile water.

Western blot analysis. Cells were scraped from the plate and lysed in RIPA buffer (10 mm Tris-HCl, 150 mm NaCl, 1% Triton X-100, 5 mm EDTA, 1% sodium deoxycholate, 0.1% SDS, 1.2% aprotinin, 5 μm leupeptin, 4 μm antipain, 1 mm phenylmethylsulphonyl fluoride, and 0.1 mm Na_3VO_4) on ice for 1 h containing a protease inhibitor mixture. Cell lysates were then centrifuged at 13,000 x g for 15 min at 4˚C, and the insoluble debris were discarded. Protein concentration of each sample was determined by BCA Protein Assay Reagent (Pierce Biotechnology, Inc., Rockford, IL, US). Then total proteins were subjected to SDS-polyacrylamide gel electrophoresis (SDS/PAGE) and transferred to nitrocellulose membranes (Millipore Corp., Billerica, MA, USA). After blocking in 5% non-fat dry milk in TBST (10 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.05% Tween-20), the membranes were incubated with primary antibodies overnight at 4˚C. The membranes were then washed four times with TBST and then incubated with HRP-conjugated secondary antibodies for 1 h at room temperature. Proteins were visualized using chemiluminescent substrate (Millipore Corp.).

Determination of apoptosis. Apoptotic cells were stained with Annexin V-FITC using an Annexin V-FITC Apoptosis Detection kit I (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. Stained cells were then analyzed by flow cytometry with a FACScan cytometer.

RNA interference. The p27 siRNA (si-p27) sequence and the scrambled siRNA control (si-ctrl) sequence were 5'-GCAACC GACGAUUCUUCUATT-3' and 5'-TTCTCCGAACGTGTC ACGTTT-3', respectively. The cells were transfected with siRNA duplexes for 48 h using Oligofectamine (Invitrogen Life Technologies) following the manufacturer's recommendations.

Tumorigenicity assay. In the experiment, NOD/SCID mice at age of 6 weeks were injected with $1x10^6$ cells in 100 μl PBS into left and right flanks, respectively. Tumor sizes were measured every few days, and tumor volumes were calculated as volume = length x width^2 x (1/2). Animals were maintained of regular food and water and after several weeks, and then were killed and tumors were harvested. All procedures were approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University.

Statistical analysis. The statistical significance of differences was evaluated by unpaired t-test, and P<0.05 was considered to indicate a statistically significant difference.

Results

Young IMR90 cells present an increase of autophagy responses than senescence cells when exposed to hypoxia. The existence of evidences that HIF-1α could induce autophagy and RB-E2F1 pathway triggers autophagy led us to explore whether HIF-1α could regulate autophagy through RB-E2F1 pathway (14,20,21). To investigate this hypothesis, young and sensescent IMR90 cells were exposed to hypoxia and then collected and analyzed. Consistent with previous findings, hypoxia induces leads to prolyl hydroxylase inhibition and stabilization of HIF-1α and the conversion of the microtubule-associated protein LC3-I to LC3-II, a biochemical marker of autophagy that is correlated with the formation of autophagosomes (Fig. 1A). To further support the observation, we performed western blot analyses of p62, which is an autophagic substrate. In response to hypoxia, the degradation of p62 was much faster in young IMR90 cells than senescent cells (Fig. 1A). Immunoblotting analyses revealed an accumulation of E2F1 in senescent IMR90 cells compared with young IMR90 cells and p27 presented an opposite expression profile to E2F1 (Fig. 1A). However, that $p16^{INK4a}$ showed upregulation as cell senescence didn't accord with the previous report that

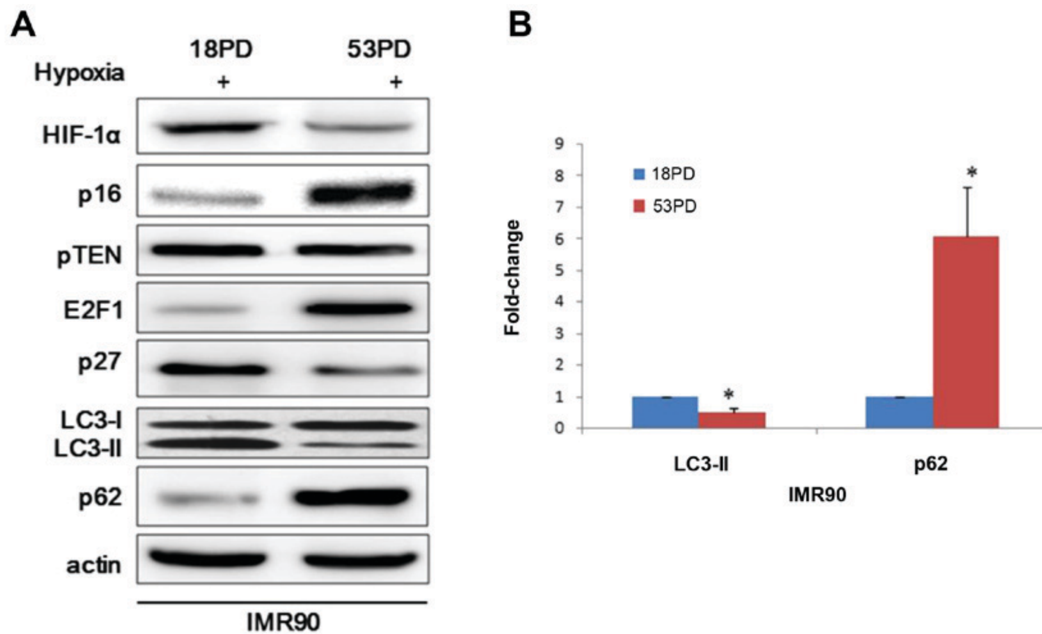

Figure 1. Young IMR90 cells present an increase of autophagy responses than senescent cells when exposed to hypoxia. (A) Young and senescent IMR90 cells were cultured in DMEM medium with 10% fetal serum and exposed to hypoxia (1% O_2) for 24 h. Cell lysates were collected and then analyzed for expression of the indicated proteins. (B) The relative intensity of LC3-II and p62 protein level was quantified with ImageJ software and normalized to the actin protein level (internal control). Values are mean ± SD of triplicate points from a representative experiment (n=3), which was repeated three times with similar results. $^{*}P<0.05$.

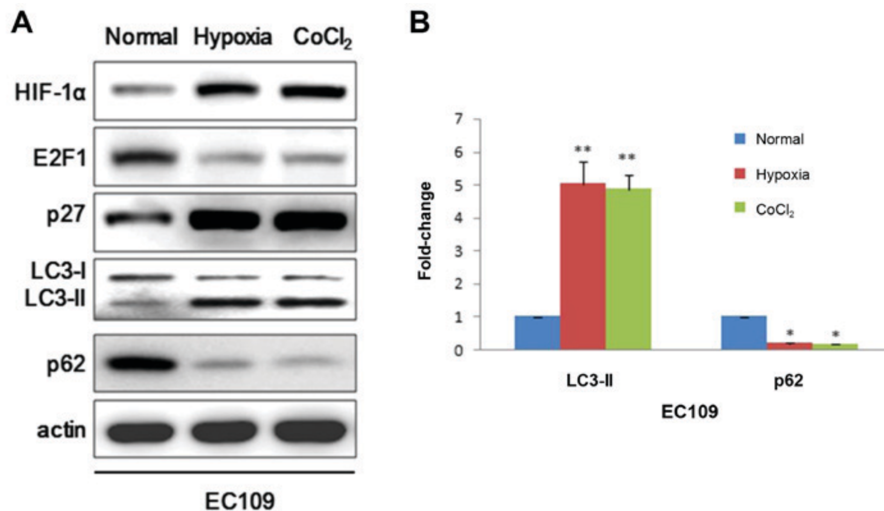

Figure 2. Hypoxia induces autophagy responses in EC109 cells. (A) EC109 cells were cultured and exposed to 20%, 1% O_2 or $CoCl_2$ for 24 h. Western blot analyzes of indicated proteins. Actin served as loading control. (B) Autophayg degree was determined by analyzing the relative intensity of LC3-II and p62 protein level described as above. Three independent experiments were analyzed. Error bars represent means ± SD (n=3) $^{*}P<0.05$, $^{**}P<0.01$.

the RB activators $p16^{INK4a}$ and p27 induce autophagy through modulating E2F1 (21). Here ImageJ software was used to analyze the ratio of LC3-II bands compared with reference bands of actin, which demonstrated that LC3-II decreased in senescent IMR90 cells relative to young IMR90 cells (Fig. 1B). Taking all the previous findings into consideration, we found that young IMR90 could produce more autophagosomes and this may indicate that young IMR90 cells fit better than senescent cell when face hypoxia.

Hypoxia induces autophagy responses in EC109 cells. We further extended this study to EC109 cells to verify whether HIF-1α could also play an important role in cancer cell. EC109 cells were exposed to 20 or 1% for 24 h. Similarly, western blot demonstrated that HIF-1α could induce autophagy by positively modulating p27 which represses E2F1 activity just in line with results in IMR90 cells (Fig. 2A). Given that hypoxia can alter a variety of metabolic parameters that could modulate autophagy activity, here we used $CoCl_2$ which is a chemical hypoxia mimetic agent to further strengthen the argument that hypoxia could induce autophagy. Consistently, $CoCl_2$ resulted in a decrease of E2F1, p62 and increases of p27 and LC3-II (Fig. 2A). LC3-II bands were also analyzed using ImageJ software (Fig. 2B). Taken together, these results support that hypoxia not only induces autophagosomes in IMR90 cells but also in EC109 cells.

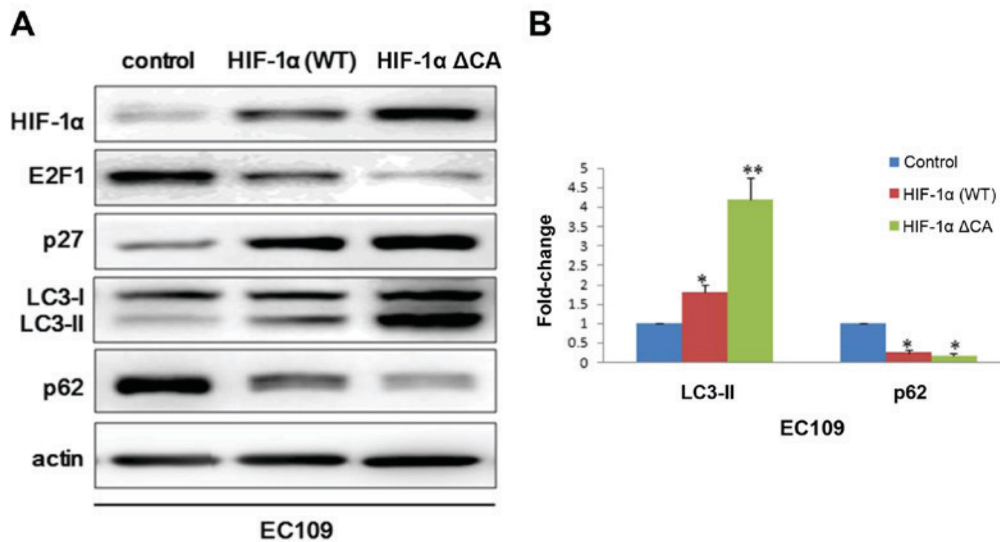

Figure 3. HIF-1α induces autophagy response in EC109 cells. (A) EC109 cells were transfected with control plasmid, HIF-1α wild type (HIF-1α WT) plasmid and HIF-1α constitutively active form of HIF-1α (HIF-1α ΔCA) plasmid. 48 h after transfection, cells were harvested and cell lysates were subjected to western blot analysis for the indicated proteins. (B) LC3-II and p62 protein level was determined by ImageJ software. Error bars represent means ± SD (n=3) $^{*}P<0.05$, $^{**}P<0.01$.

HIF-1α induces autophagy response in EC109 cells. In order to further explore the role of HIF-1α in autophagy. Vector control plasmid, HIF-1α wild type plasmid and HIF-1α constitutive active plasmid were transfected into EC109 cells. Consistent with above results, we observed that p27 expression was significant higher in HIF-1α ΔCA group than HIF-1α wild type group and blank control group. While E2F1 and p62 presented an opposite expression profile with p27 (Fig. 3A). To further assess autophagosome maturation, we performed degree scanning of LC3-II which is an autophagic marker (Fig. 3B).

P27 silence inhibits hypoxia induced autophagy. Because E2F1 activity is negatively regulated by CDKIs, such as p16 and p27 (22), and previous results demonstrated that p16 and p27 could induce autophagy in some cell lines. To this end, we presumed that HIF-1α may modulate autophagy via p16 and p27 pathways. Whereas p16 has a different expression profile with p27, so we discreetly think p27 is the downstream target in HIF-1α induced autophagy. We then challenged the autophagy mechanism by down-modulating p27 when in hypoxia condition. As expected, silencing p27 in EC109 cells decreased HIF-1α mediated processing of formation of LC3-II and increased p62 expression (Fig. 4A and B). Consistently, immunoassay showed E2F1 appeared high expression when p27 silenced (Fig. 4A). Because expression of E2F1 induces apoptosis (23), we asked whether the cells that were transduced with siRNA against p27 were undergoing cell death through apoptosis. Therefore, Bcl-2 which is an autophagy inhibitor and upregulated by E2F1 was examined (24,25). Here we showed that repression of p27 lead to up-modulating of Bcl-2 and cleavage of PARP (Fig. 4A), contributing to the triggering of apoptosis. To further determine whether the cause of the cell death was apoptosis, we used flow cytometry to examine the ability of the cells binding to Annexin V. We found that p27 silence caused about 32% of the cells to undergo apoptosis, much higher than the control group (Fig. 4C and D). Thus, we conclude that HIF-1α plays a role in the control of autophagy via regulating p27-E2F1 pathway.

HIF-1α promotes tumor cell proliferation and tumorigenesis in vivo. Given several lines of evidence indicate that HIF-1α function contributes to tumor growth, we next examined the effect of HIF-1α on cell proliferation and tumor growth. To determine whether HIF-1α rendered growth advantage to tumor cell *in vivo*, we performed tumor xenograft studies. EC109 cell lines with stable expression of vector control and HIF-1α ΔCA were injected into the left and right flanks of mude mice and tumor cell growth was monitored over a period of 30 days. We found that cells expressing HIF-1α% ΔCA grew at a faster rate than that the cells expressing vector control. Representative kinetics of tumor growth are shown in Fig. 5B. Therefore, these results demonstrate that HIF-1α promotes cell proliferation and tumor growth by inducing autophagy in hypoxia environment.

Discussion

As we all known autophagy is an evolutionarily conserved process that occurs as a physiological process in normal cells at a basal level to assure cellular homeostasis, or as a strategic survival mechanism under hypoxia, stress and nutrient deprivation conditions (3,26). Though various kinds of research have focused on the mechanisms of autophagy and several gene have been identified that could take part in the autophagy process. However, the autophagy mechanisms that are involved in cancer remain largely obscure. As we know that IMR90 cells have served as a non tumor control cell line in a wide variety of studies and esophageal cancer is one of the most malignant cancers in China (27,28). So in this study, we used IMR90 cells and esophageal carcinoma cell line EC109 cells to investigate the role of autophagy in normal human cell and tumor cell. Thereafter, we identified HIF-1α regulates autophagy via modulating p27-E2F1 pathway and we uncover

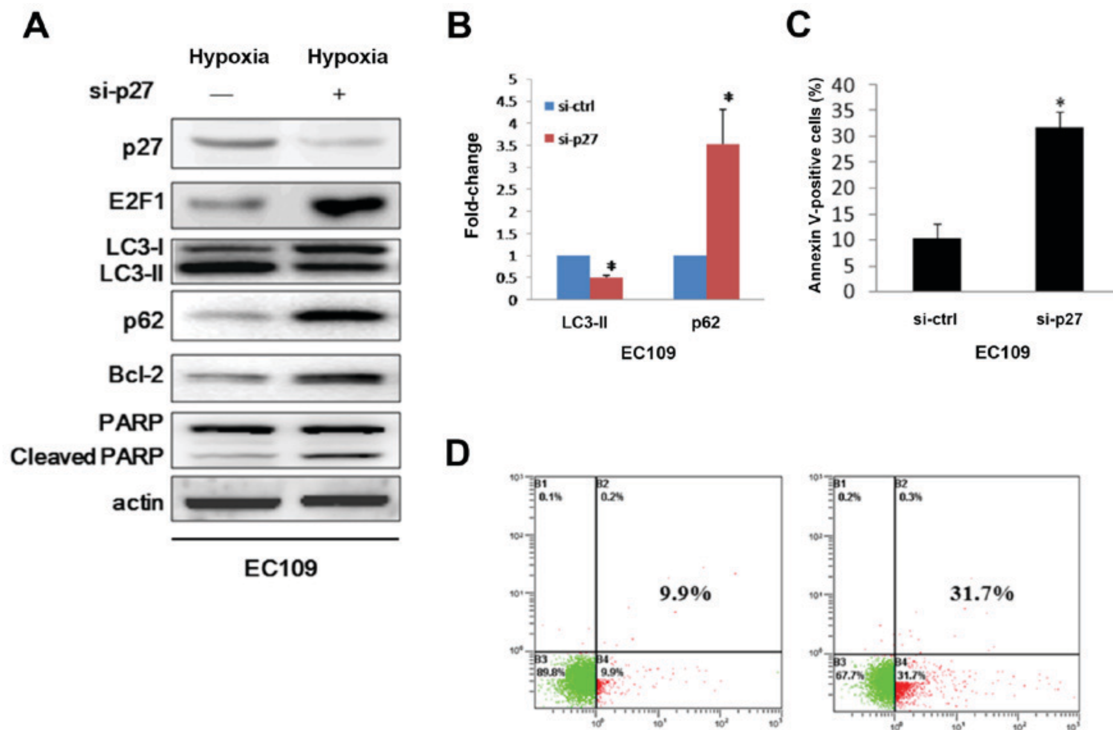

Figure 4. P27 silence inhibits hypoxia induced autophagy. (A) EC109 cells were mock or p27 siRNA transfected according to the manufacturer's protocol. Then the mock group was exposed to 20% O_2, while p27 siRNA transfected group was cultured in 1% O_2 for 24 h. Thereafter, total proteins from the cell lysates were collected and analyzed for the expression of indicated proteins. (B) The ratio of LC-3 II bands were analyzed by ImageJ software. (C and D) Cells were treated as described in A and were stained with Annexin V and analyzed by flow cytometry. Columns, mean from at least three independent experiments. Error bars represent means ± SD (n=3). *P<0.05.

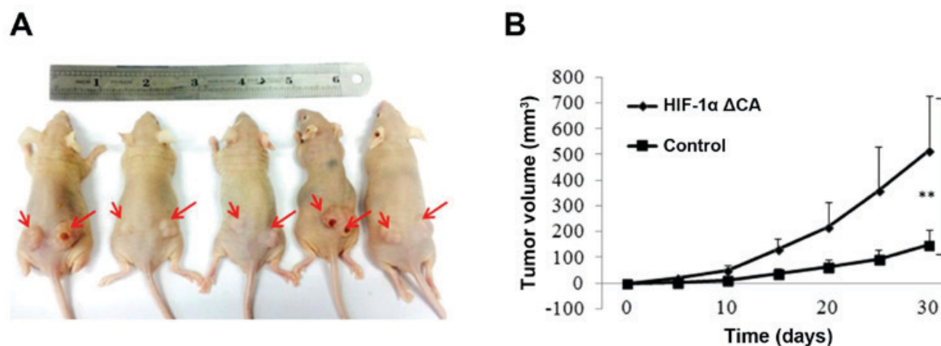

Figure 5. HIF-1α promotes tumor cell proliferation and tumorigenesis *in vivo*. (A) HIF-1α contributes xenograft tumor growth. EC109 cell lines ($1x10^6$) with control and HIF-1α ΔCA were injected subcutaneously into left and right flanks of nude mice. At 30 days after injection, tumors were extracted and photographed. (B) Tumor diameters were measured at the indicated time points and tumor volumes were calculated. **P<0.01.

a previously unidentified connection between HIF-1α and autophagy for the first time.

HIF-1α has been found to be the main reason for malignant tumor survival in hypoxia (29,30). As a transcriptional activator, HIF-1α can induce the expression of kinds of target genes, which promote angiogenesis, enhance hypoxia tolerance (31). Here, we found HIF-1α can induce autophagy through p27-E2F1 pathway under hypoxia which could degrade cell constituents and supply energy for cell survival. Our finding confirms the mechanism that autophagy is a surveillance mechanism used by normal cells to protect them from transformation to malignancy by removing damaged organelles and reducing reactive oxygen species. Besides, the autophagy mechanisms are also involved in cancer cell.

As we known E2F1 activity is negatively regulated by CDKIs, such as $p16^{INK4a}$ and p27 and the E2F1 pathway is crucial in regulating cell growth and apoptosis. So we asked whether suppression of E2F1 by CKDIs would result in autophagy. Here, we report that p27 positively regulates autophagy by repressing E2F1 activity in IMR90 cells, while $p16^{INK4a}$ don't take part in this pathway. This may coincide with that p27 is a downstream gene of HIF-1 under lack of energy conditions (32). As the expression of E2F1 induces apoptosis and we discovered that p27 silence inhibits HIF-1α induced autophagy. Our results conclude that p27 silence caused a higher percent of the cells to undergo apoptosis. Thus, our results suggest that HIF-1α regulates autophagy through p27-E2F1 pathway. P27 and E2F1

proteins play opposite roles in the control of both autophagy and apoptosis.

In summary, we report here that young IMR90 cells present an increased of autophagy responses than senescence cells when exposed to hypoxia and HIF-1α could also induce autophagy in EC109 cells through p27-E2F1 pathway. Ablation of p27 inhibits HIF-1α induced autophagy and promotes E2F1 induced apoptosis. Our results may shed light on understanding the compulsive biological mechanism of autophagy.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (grant no. 81501200), the China Postdoctoral Science Foundation (funded project no. 2015M572122) and the Project of Medical Science and Technology Research of Henan Provincial Health and Family Planning Commission (grant no. 201503029).

References

1. Mizushima N, Levine B, Cuervo AM and Klionsky DJ: Autophagy fights disease through cellular self-digestion. Nature 451: 1069-1075, 2008.
2. Levine B and Kroemer G: Autophagy in the pathogenesis of disease. Cell 132: 27-42, 2008.
3. Cao J, Ying M, Xie N, Lin G, Dong R, Zhang J, Yan H, Yang X, He Q and Yang B: The oxidation states of DJ-1 dictate the cell fate in response to oxidative stress triggered by 4-hpr: Autophagy or apoptosis? Antioxid Redox Signal 21: 1443-1459, 2014.
4. Rubinsztein DC: The roles of intracellular protein-degradation pathways in neurodegeneration. Nature 443: 780-786, 2006.
5. Yang Z and Klionsky DJ: Eaten alive: A history of macroautophagy. Nat Cell Biol 12: 814-822, 2010.
6. Mijaljica D, Prescott M and Devenish RJ: Microautophagy in mammalian cells: Revisiting a 40-year-old conundrum. Autophagy 7: 673-682, 2011.
7. Jiang P and Mizushima N: Autophagy and human diseases. Cell Res 24: 69-79, 2014.
8. White E: Deconvoluting the context-dependent role for autophagy in cancer. Nat Rev Cancer 12: 401-410, 2012.
9. Burada F, Nicoli ER, Ciurea ME, Uscatu DC, Ioana M and Gheonea DI: Autophagy in colorectal cancer: An important switch from physiology to pathology. World J Gastrointest Oncol 7: 271-284, 2015.
10. Cecconi F and Jäättelä M: Targeting ions-induced autophagy in cancer. Cancer cell 26: 599-600, 2014.
11. Shintani T and Klionsky DJ: Autophagy in health and disease: A double-edged sword. Science 306: 990-995, 2004.
12. Sena P, Mariani F, Mancini S, Benincasa M, Magnani G, Pedroni M, Palumbo C and Roncucci L: Autophagy is upregulated during colorectal carcinogenesis, and in DNA microsatellite stable carcinomas. Oncol Rep 34: 3222-3230, 2015.
13. Shelby SJ, Angadi PS, Zheng QD, Yao J, Jia L and Zacks DN: Hypoxia inducible factor 1α contributes to regulation of autophagy in retinal detachment. Exp Eye Res 137: 84-93, 2015.
14. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ and Semenza GL: Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem 283: 10892-10903, 2008.
15. Zhu H, Wang D, Zhang L, Xie X, Wu Y, Liu Y, Shao G and Su Z: Upregulation of autophagy by hypoxia-inducible factor-1α promotes EMT and metastatic ability of $CD133^+$ pancreatic cancer stem-like cells during intermittent hypoxia. Oncol Rep 32: 935-942, 2014.
16. Wang GL, Jiang BH, Rue EA and Semenza GL: Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci USA 92: 5510-5514, 1995.
17. Blagosklonny MV: Antiangiogenic therapy and tumor progression. Cancer cell 5: 13-17, 2004.
18. Semenza GL: HIF-1 and mechanisms of hypoxia sensing. Curr Opin Cell Biol 13: 167-171, 2001.
19. Seeber LM, Horrée N, Vooijs MA, Heintz AP, van der Wall E, Verheijen RH and van Diest PJ: The role of hypoxia inducible factor-1alpha in gynecological cancer. Crit Rev Oncol Hematol 78: 173-184, 2011.
20. Tittarelli A, Janji B, Van Moer K, Noman MZ and Chouaib S: The selective degradation of synaptic connexin 43 protein by Hypoxia-induced autophagy impairs natural killer cell-mediated tumor cell killing. J Biol Chem 290: 23670-23679, 2015.
21. Jiang H, Martin V, Gomez-Manzano C, Johnson DG, Alonso M, White E, Xu J, McDonnell TJ, Shinojima N and Fueyo J: The RB-E2F1 pathway regulates autophagy. Cancer Res 70: 7882-7893, 2010.
22. Chen T, Xue L, Niu J, Ma L, Li N, Cao X, Li Q, Wang M, Zhao W, Li G, *et al*: The retinoblastoma protein selectively represses E2F1 targets via a TAAC DNA element during cellular senescence. J Biol Chem 287: 37540-37551, 2012.
23. Polager S and Ginsberg D: E2F-at the crossroads of life and death. Trends Cell Biol 18: 528-535, 2008.
24. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD and Levine B: Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 122: 927-939, 2005.
25. Luo S and Rubinsztein DC: Apoptosis blocks Beclin 1-dependent autophagosome synthesis: An effect rescued by Bcl-xL. Cell Death Differ 17: 268-277, 2010.
26. Chagin AS: Effectors of mTOR-autophagy pathway: Targeting cancer, affecting the skeleton. Curr Opin Pharmacol 28: 1-7, 2016.
27. Pei Y, Wang P, Liu H, He F and Ming L: FOXQ1 promotes esophageal cancer proliferation and metastasis by negatively modulating CDH1. Biomed Pharmacother 74: 89-94, 2015.
28. Kirschner K, Samarajiwa SA, Cairns JM, Menon S, Pérez-Mancera PA, Tomimatsu K, Bermejo-Rodriguez C, Ito Y, Chandra T, Narita M, *et al*: Phenotype specific analyses reveal distinct regulatory mechanism for chronically activated p53. PLoS Genet 11: e1005053, 2015.
29. Vaupel P: The role of hypoxia-induced factors in tumor progression. Oncologist 9 (Suppl 5): S10-S17, 2004.
30. Unruh A, Ressel A, Mohamed HG, Johnson RS, Nadrowitz R, Richter E, Katschinski DM and Wenger RH: The hypoxia-inducible factor-1 alpha is a negative factor for tumor therapy. Oncogene 22: 3213-3220, 2003.
31. Clarke HJ, Chambers JE, Liniker E and Marciniak SJ: Endoplasmic reticulum stress in malignancy. Cancer Cell 25: 563-573, 2014.
32. Zhu Y, Zhao T, Itasaka S, Zeng L, Yeom CJ, Hirota K, Suzuki K, Morinibu A, Shinomiya K, Ou G, *et al*: Involvement of decreased hypoxia-inducible factor 1 activity and resultant G1-S cell cycle transition in radioresistance of perinecrotic tumor cells. Oncogene 32: 2058-2068, 2013.