Research Article

IDH1 Mutation Induces HIF-1α and Confers Angiogenic Properties in Chondrosarcoma JJ012 Cells

Xiaoyu Hu,1,2 Luyuan Li,3,4 Josiane E. Eid,3,4 Chao Liu,2 Jinming Yu,1,2 Jinbo Yue,2 and Jonathan C. Trent3,4

1Department of Oncology, Renmin Hospital of Wuhan University, Wuhan, China
2Department of Radiation Oncology, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, Shandong, China
3Department of Medicine, Division of Medical Oncology, University of Miami Miller School of Medicine, Miami, USA
4Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, USA

Correspondence should be addressed to Jinming Yu; sdyujinming@163.com, Jinbo Yue; jbyue@sdfmu.edu.cn, and Jonathan C. Trent; jtrent@med.miami.edu

Xiaoyu Hu and Luyuan Li contributed equally to this work.

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Chondrosarcoma is a group of primary bone cancers that arise from transformed cells of chondrocytic lineage. Tumor recurrence and metastasis are devastating for patients with chondrosarcoma since there are no effective treatment options. IDH mutations occur in over 50% of tumors from patients with conventional or dedifferentiated chondrosarcoma and represent an attractive target for therapy. However, their role in the pathogenesis of chondrosarcoma remains largely unknown. In this study, we sought to determine the association of IDH mutation and HIF-1α in chondrosarcoma. We used the chondrosarcoma JJ012 cell line and its derived CRISPR/Cas9 mutant IDH1 (IDH1 mut) knockout (KO) cells. RNA-Seq data analysis revealed downregulation of several HIF-1α target genes upon loss of IDH1 mut. This was associated with reduced HIF-1α levels in the IDH1 mut KO cells and tumors. Loss of IDH1 mut also attenuated the expression of angiogenic markers in tumor tissues and abrogated the angiogenic capacity of JJ012 cells. Moreover, we observed that exogenous expression of HIF-1α significantly promoted anchorage-independent colony-formation by IDH1 mut KO cells. These results suggest IDH1 mutation confers angiogenic and tumorigenic properties of JJ012 cells by inducing HIF-1α. Thus, the HIF pathway represents a promising candidate for combinatorial regimens to target IDH1 mutated chondrosarcomas.

1. Introduction

Chondrosarcomas constitute a heterogeneous group of primary bone cancers characterized by the formation of a hyaline cartilaginous matrix. Following osteosarcoma, chondrosarcoma is the second most common bone malignancy, accounting for 20% to 27% of primary bone tumors [1, 2]. Approximately 85% of chondrosarcomas are the conventional subtypes which can be further classified into central, peripheral, and periosteal lesions. The remaining 10–15% consist of rare subtypes including dedifferentiated, mesenchymal, clear cell, and myxoid chondrosarcoma. Chondrosarcomas are notoriously resistant to chemotherapy and radiotherapy, and surgery is the backbone treatment for most localized tumors [2, 3]. Chondrosarcomas tend to recur with more aggressive behavior than the original neoplasm following initial tumor resection. As a result, many patients develop metastatic disease which is nearly uniformly fatal. Due to lack of effective treatment strategies for recurrent or metastatic chondrosarcoma, high-grade conventional and dedifferentiated chondrosarcomas have poor prognosis [1, 4]. Current studies focus on clarifying the link between molecular events and pathogenesis of this malignancy and developing new molecularly targeted therapies for advanced diseases.
Isocitrate dehydrogenase (IDH) mutation is among one of the promising therapeutic targets. IDH1/2 mutations were found in 71% of conventional chondrosarcomas and 57% of dedifferentiated chondrosarcomas [4, 5], as well as in gliomas and acute myeloid leukemia [6, 7], suggesting a potential role for aberrant IDH function in the pathogenesis of these malignancies. IDHs normally convert isocitrate to α-ketoglutarate (α-KG). However, mutant IDHs lose the ability to catalyze this reaction but instead gain a neomorphic function of reducing α-KG to D-2-hydroxyglutarate (D-2HG), which has been reported to accumulate at high levels in IDH1/2-mutated tumors [8, 9]. D-2HG and α-KG are structurally similar. Thus, accumulated D-2HG is thought to act as an oncometabolite through the inhibition of various α-KG-dependent enzymes including the TET family of 5-methylcytosine hydroxylases, JumonjiC domain-containing histone demethylases (JHDMS), and the Prolyl Hydroxylase Domain-Containing Proteins (PHDs) [9–11].

HIF-1α, a key hypoxia-inducible transcription factor, is associated with tumor development as it functions as a master regulator of genes involved in angiogenesis, glucose metabolism, and other cellular pathways [12]. HIF-1α overexpression is correlated with disease progression, chemoresistance, and increased patient mortality in certain cancers [13–15]. The stability and transcriptional activity of HIF-1α are regulated by PHDs (PHD1, PHD2, and PHD3). Under normal oxygen conditions, PHDs utilize α-KG and O2 to hydroxylate a conserved proline in HIF-1α, leading to Von Hippel-Lindau- (VHL-) mediated ubiquitination and subsequent proteasomal degradation of HIF-1α [16, 17]. In hypoxic conditions, however, these hydroxylative events cannot proceed efficiently, resulting in accumulation of HIF-1α [18]. Interestingly, studies examining the effects of D-2HG on PHDs and HIF-1α in IDH-mutant gliomas and leukemias have yielded conflicting results. It has been reported that D-2HG competitively inhibits the activity of PHDs as mentioned above and thus leads to increased levels of HIF-1α [18, 19]. Conversely, D-2HG was shown to act as an activator rather than an inhibitor of PHDs, ultimately leading to decreased levels of HIF-1α [20, 21]. In contrast to the emerging knowledge in gliomas and leukemias, little is known regarding the effect of IDH mutation or D-2HG on HIF-1α activity in chondrosarcoma. Understanding their relationship would have great clinical importance in terms of developing novel targeted therapies for advanced chondrosarcomas.

In this study, we sought to determine the potential association of IDH mutation and HIF-1α in chondrosarcoma. We employed the IDH1-mutant chondrosarcoma JJ012 cell line, CRISPR/Cas9 mutant IDH1 (IDH1mut) knockout (KO) JJ012 clones, and their derived xenografts. We found that CRISPR/Cas9 knockout of IDH1mut reduced HIF-1α levels in vitro and in vivo, leading to downregulation of HIF-1α target genes. Loss of IDH1mut also decreased the expression of angiogenic markers in tumors and attenuated the angiogenic capacity of JJ012 cells. Moreover, we observed restoring HIF-1α levels with exogenous expression significantly enhanced the anchorage-independent growth of IDH1mut KO cells. These results suggest IDH1 mutation confers tumorigenic and angiogenic properties by inducing HIF-1α in a JJ012 chondrosarcoma model.

2. Materials and Methods

2.1. Cell Culture. The human chondrosarcoma JJ012 and human chondrocyte C28 cell lines were kindly provided by Dr. Joel Block and Dr. Karina Galoian, respectively. JJ012 harbors a monoallelic IDH1 R132G mutation while C28 carries wildtype IDH1 (IDH1wt). JJ012 cells were cultured in RPMI-1640 medium (Lonza) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin. C28 cells were grown in 1:1 DMEM/F12 medium (HyClone) supplemented with 10% FBS and 1% Penicillin/Streptomycin. Human umbilical vein endothelial cells (HUVECs) were obtained from Thermo Fisher Scientific and grown in Endothelial Cell Growth Medium 2 (EGM-2) (PromoCell) on 0.1% gelatin-coated plates. Cells were maintained at 37°C in a humidified air with 5% CO2.

2.2. IDH1 Knockout by CRISPR/Cas9 Technology. Knockout of IDH1mut was achieved by the CRISPR/Cas9 system. The CRISPR/Cas9 plasmid products were purchased from Santa Cruz Biotechnology. Details of transfection, selection, and single-cell colonies propagation were previously described [22].

2.3. Measurement of D-2HG. Quantitative analyses of D-2HG were conducted by high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) with single-reaction monitoring (SRM) scans. This was performed at MtoZ Biolabs (Boston, MA, USA).

2.4. Tumor Tissues. Tumor samples were obtained from mice-bearing chondrosarcoma xenografts which were derived from JJ012 parental and IDH1mut KO cells, as previously described. All animal experiments were performed in compliance with the University of Miami Institutional Animal Care and Use Committee (IACUC)-approved protocol (No. 19-079).

2.5. RNA-Seq and Ingenuity Pathway Analysis (IPA). RNA-Seq and IPA were described with details previously [22].

2.6. Quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR). Total RNA was extracted using the miRNeasy Mini Kit (Qiagen) according to the manufacturer’s protocol. cDNA was synthesized from 2μg of total RNA using iScript a cDNA synthesis kit (Bio-Rad) supplemented with 2-mercaptoethanol in a 40μl total reaction volume. qRT-PCR was set up with 20x TaqMan probes, 2μl of 1:5 diluted cDNA and TaqMan universal PCR Master Mix (Thermo Fisher Scientific) in 20μl total volume. Samples were run in triplicate on a Bio Rad CFX-96 real time PCR system. Gene expression levels were calculated using the 2–ΔΔCt method [23]. Gene-specific TaqMan primers/probe sets include GAPDH (internal normalization control), VEGFA, VEGFC, EDN1, and SLC2A3.

2.7. Western Blotting. Cells were lysed in a Laemmli sample buffer (Bio-Rad) supplemented with 2-mercaptoethanol (Sigma-Aldrich). The lysates were centrifuged at
were then maintained in regular incubator with 21% O₂, or
10% FBS overlaid on a lower layer of a 0.5% basal agar. Cells
plated in a 0.3% top layer soft agar in RPMI-1640 with
(2 and incubated with 1 ml of cell supernatant secretome for
MgCl₂-supplemented PBS, and
(0.5 μg/ml) for 30 min, rinsed with 1 mM CaCl₂ and 0.5 mM
SLC2A3 in the IDH1 mut KO cells was further
established HIF-1α target genes including VEGFA, VEGFC,
EDN1 and SLC2A3 in the IDH1 mut KO cells was further
confirmed by qRT-PCR (Figure 1(d)). These results indicate
that IDH mutation is associated with activation of the HIF-1α
signaling pathway.

To be noted, both IDH1 wt and IDH1 mut were knocked
out in our cell model. To confirm that it is the loss of IDH1-
mut allele rather than that of IDH1 wt is responsible for the
downregulation of the HIF-1α target genes, we utilized C28
cells, an immortalized human chondrocyte cell line that
expresses IDH1 wt only [24]. A pool of C28 IDH1 wt KO cells
with markedly reduced IDH1 levels was created using the
CRISPR/Cas9 technique (Figure 1(e)). qRT-PCR analysis
revealed comparable expression of the aforementioned
HIF-1α target genes between C28 parental and IDH1 wt
KO cells (Figure 1(f)), suggesting that loss of the IDH1 mut
rather than of the IDH1 wt allele caused the downregulation of the
HIF-1α target genes in JJ012 cells.

3.2. Loss of IDH1 mut Reduces HIF-1α Levels in JJ012 Cells and
Tumor Tissues. HIF-1α protein levels are regulated by PHDs
which destabilize the angiogenic transcription factor by
post-translational proline hydroxylation under normoxic
conditions. It has been reported that D-2HG competitively
inhibits PHDs due to its structural similarity to α-KG,
thereby causing accumulation of HIF-1α in IDH1-mutant
glioma cells [19]. In our model, D-2HG production was
almost completely suppressed in JJ012 IDH1 mut KO clones
[22]. Therefore, it is rational to inquire whether the down-
regulation of HIF-1α target genes in the IDH1 mut KO cells
is attributed to HIF-1α inhibition as a result of IDH1 mut loss
and reduced D-2HG production. Indeed, HIF-1α expression
appeared to be significantly decreased in the two IDH1 mut
KO JJ012 cell lines compared to their parental control under

14,000 rpm for 10 min at 4° C. The supernatants were col-
clected and denatured at 95° C for 10 min. Equal protein
lysates were separated on 4-20% Mini-PROTEIN TGX pre-
cast gels (Bio-Rad) and transferred to nitrocellulose mem-
branes (Pall Corporation). The following antibodies were
used: anti-IDH1 (1:1000, Abcam, ab172964), anti-HIF-1α
(0.5 μg/ml, RD Systems, NB100-105), and anti-β-actin
(1:5000, Cell Signaling).

2.9. Immunohistochemistry (IHC). Immunohistochemical
analysis was performed on 5-μm sections cut from forma-
lin-fixed, paraffin-embedded samples utilizing antibodies
against CD31 (Servicebio, GB11063-2), HIF-1α (Bioworld,
BS3514), VEGFA (Abcam, ab52917), and IDH1 (Abcam,
ab172964) following a standard protocol. A semiquantitative
evaluation method was applied as follows: the score obtained
by the percentage of positive cells ((0% = 0; 1 – 25% = 1, 26
– 50% = 2, 51 – 75% = 3, and >75% = 4) was multiplied by
the score obtained by the staining intensity (no staining = 0 ,
weak staining = 1, moderate staining = 2, and strong
staining = 3). Scoring was evaluated by investigators who
were blinded to the information of research subjects.

2.10. Vascular-Endothelial Tube Formation Assay. JJ012
parental and IDH1 mut KO cells were cultured with serum-
supplemented RPMI-1640 overnight, followed by a 24 h
starvation in serum-free medium. Secretome derived from
the culture supernatant was then collected and centrifuged
at 1200 rpm for 5 min to remove debris. The vascular-
endothelial tube formation assay was performed with
HUVEC cells in 24-well plates pre-coated with growth
endothelial tube formation assay was performed with

3. Results

3.1. Loss of IDH1 mut Leads to Downregulation of HIF-1α
Target Genes in JJ012 Cells. In this study, we utilized the
human chondrosarcoma JJ012 cell line which harbors an
endogenous IDH mutation, and its derived two CRISPR/
Cas9 IDH1 mut KO clones. Depletion of the IDH1 protein
and reduced D-2HG levels in the two IDH1 mut KO clones
are shown in Figures 1(a) and 1(b), respectively. To begin
to understand the role of IDH mutation in chondrosarcoma
tumorigenesis, we initially conducted an RNA-Seq analysis
of JJ012 parental cells and its two IDH1 mut KO clones. The
RNA-Seq analysis revealed an association between IDH
mutation and aberrant activation of integrin signaling in
chondrosarcoma [22]. Interestingly, in addition to mediators
of cell adhesion and integrin-related pathways, we found in
the transcriptome of JJ012 IDH1 mut KO cells many genes
known to be involved in vasculogenesis. The downregulated
HIF-1α target genes detected in both IDH1 mut KO clones are
shown in Figure 1(c). These genes are implicated in glucose
metabolism (SLC2A3, LDHA and LDHB) and angiogenesis
(VEGFA and VEGFC). Downregulation of several well-
established HIF-1α target genes including VEGFA, VEGFC,
EDN1 and SLC2A3 in the IDH1 mut KO cells was further
confirmed by qRT-PCR (Figure 1(d)). These results indicate
that IDH mutation is associated with activation of the HIF-1α
signaling pathway.

Disease Markers
Figure 1: Continued.
We then proceeded to ask whether HIF-1α was similarly regulated in vivo. For this we measured IDH1 expression in the chondrosarcoma xenografts derived from parental and IDH1mut KO JJ012 cells that we have previously established [22]. IHC analysis revealed significant reduction of IDH1 in the IDH1mut KO tumors (Figure 2(b)). Importantly, HIF-1α levels were concomitantly reduced in the same tumors, thus confirming our in vitro findings. The staining score showed that HIF-1α expression was reduced by 70%-80% in the IDH1 mut KO tumors compared to parental controls (Figure 2(c)). These results demonstrate that knockout of IDH1mut downregulates HIF-1α in vitro and in vivo, thus supporting the concept that IDH1 mutation promotes HIF-1α stabilization and its downstream signaling in our JJ012 chondrosarcoma model.

3.3. IDH1 Mutation Confers Angiogenic Properties in JJ012 Chondrosarcoma Cells. Angiogenesis represents an essential step in tumor proliferation, expansion, and metastasis, thus contributing to the pathology of virtually all human cancers. HIF-1α is a subunit of HIF-1, an oxygen-dependent transcriptional activator, which plays crucial roles in tumor angiogenesis and mammalian development [12]. HIF-1 activates transcription of genes encoding angiogenic growth factors which are secreted by hypoxic cells and promote
endothelial cell growth [25]. Given the apparent association of HIF-1α expression with IDH1 mutation, we asked whether such mutation confers angiogenic properties on the chondrosarcoma JJ012 cells. Interestingly, functional analysis of the transcriptome in JJ012 IDH1mut KO cells identified angiogenesis as one of the most prominently regulated programs (adjusted p = 1.02E−06) in chondrosarcoma. Upon further examination of angiogenic markers by IHC, we observed that CD31 and VEGFA expression in JJ012 IDH1mut KO cell-derived tumors was markedly reduced when compared to parental cell-derived tumors (Figures 3(a) and 3(b)). These findings suggest a functional association of IDH1 mutation with the HIF-1α-driven angiogenic pathway. To further assess this functional link, we conducted vascular-endothelial tube formation assays using HUVECs incubated with the secretome of JJ012 parental or IDH1mut KO cells. We found that culturing with the DH1mut KO secretome significantly inhibited HUVECs ability to form vascular tubes and reduced capillary tube length by over 20% (p < 0.05) (Figure 3(c)). These findings indicate that IDH1-mutant JJ012 chondrosarcoma cells produce a secretome highly capable of stimulating angiogenesis and promoting tumor growth.

3.4. HIF-1α Contributes to Tumorigenicity of IDH1 Mutation in JJ012 Cells. HIF-1α regulates gene expression in critical pathways that drive tumorigenesis [25]. Thus, we endeavored to determine whether HIF-1α contributes to the oncogenic properties of IDH mutation in the JJ012 chondrosarcoma cells. One of the defining criteria of tumorigenicity is anchorage-independent cell growth [26]. In our previous study, we demonstrated that loss of IDH1mut attenuated the tumorigenic potential of chondrosarcoma cells. In particular, deletion of IDH1mut led to a marked reduction in JJ012 capacity for anchorage-independent growth in soft agar [22]. To verify whether HIF-1α contributes to the observed promotion of colony formation by IDH mutation, we performed this assay under conditions of hypoxia (1% O2) and normoxia (21% O2) using JJ012 parental and IDH1-mut KO cells. We observed that hypoxia caused a dramatic increase in colony numbers in the IDH1 mut KO groups (over 60%; p < 0.05), compared with those grown under normoxic conditions (Figure 4(a)). A similar pattern was also seen in the parental cells (Figure 4(a)). Notably, growth in hypoxia appeared to abolish the previously reported difference in colony formation between the parental and IDH1mut KO JJ012 cells performed in a normoxic atmosphere (22). HIF-1α levels under both conditions were analyzed by immunoblotting. As expected, incubation with 1% O2 stimulated HIF-1α expression to comparable levels in all three cell groups (Figure 4(b)). Together, these results suggest that IDH1 mutation contributes to JJ012 cells oncogenic functions, at least in part through HIF-1α activation.

![Figure 2: Loss of IDH1mut suppresses HIF-1α in JJ012 cells and tumor tissues. (a) Immunoblots show HIF-1α expression in JJ012 parental and IDH1mut KO cells; immunohistochemical (IHC) images show cytoplasmic IDH1 (b) and nuclear HIF-1α (c) expression in parental and IDH1mut KO JJ012-derived xenografts. IHC staining was quantified with intensity scores as detailed in Materials and Methods. Scale bars are 50 μm. Data represent mean ± SEM of values from four random fields. **p < 0.01.](image-url)
4. Discussion

Tumor recurrence and metastasis are major challenges in the treatment of chondrosarcomas. Metastatic chondrosarcoma has a dismal prognosis due to lack of effective systemic therapies. IDH1/2 mutations have been frequently found in chondrosarcoma and have become an attractive target for IDH-mutant advanced chondrosarcomas. By means of drug inhibition and CRISPR/Cas9 knockout of IDH1\textsuperscript{mut}, our previous studies have implicated IDH mutation in chondrosarcoma tumorigenicity, \textit{in vitro} and \textit{in vivo} [22, 24]. However, the underlying mechanism remains largely unknown. It has been proposed that D-2HG at high levels acts as an oncometabolite and exerts some potential pro-tumorigenic effects by competitively inhibiting α-KG-dependent enzymes such as PHDs which regulate HIF-1α stability. Studies have shown IDH mutations compromise the activity of PHD and stabilize HIF-1α in glioma cells under normoxic conditions, leading to inappropriate activation of its target genes [18, 19]. Moreover, HIF-1α and its target genes such as Glut1, VEGF, and PGK1 are also upregulated in the brains of IDH1 R132H knock-in mice [27]. Consistently, in our study, RNA-Seq data analysis of chondrosarcoma JJ012 cells revealed downregulation of several HIF-1α target genes upon loss of IDH1-mut. This is correlated with reduced HIF-1α levels in these IDH1\textsuperscript{mut} KO cells and tumors compared with their parental controls. These findings are suggestive of a similar association between IDH mutation and HIF-1α induction in chondrosarcoma cells.

HIF1α is a key component of HIF1, a transcription factor that senses low cellular oxygen levels and regulates the expression of genes implicated in glucose metabolism, angiogenesis, and other signaling pathways that are critical to tumor growth. Increased expression of HIF-1α is closely associated with tumor progression in various cancers [28, 29]. Interestingly, two studies evaluated the expression of HIF-1α in cartilage tumors and suggested that HIF-1α expression was significantly correlated with shorter disease-free survival in chondrosarcoma [30, 31]. To determine whether HIF1-1α is a contributor in IDH mutation-driven tumorigenesis of chondrosarcoma, we examined the capacity of JJ012 parental and IDH1\textsuperscript{mut} KO cells for anchorage-independent growth under normoxia and hypoxia conditions in a soft-agar colony formation assay. We observed
that IDH1\textsuperscript{mut} knockout cells formed less colonies than parental cells under normoxia condition, but exogenous induction of HIF-1\(\alpha\) significantly boosted the colony-forming capacity of these cells to a degree that is comparable with that of the parental cells. This suggests that activation of HIF-1\(\alpha\) signaling is involved in the tumorigenic activity of IDH1\textsuperscript{mut} in vitro. Of note, our previous study has shown that loss of IDH1\textsuperscript{mut} led to a marked attenuation of chondrosarcoma tumor formation and D-2HG production in a xenograft model [22]. Since anchorage-independent growth is tightly correlated with tumorigenic potential in vivo, it is conceivable that the attenuated HIF-1\(\alpha\) signaling caused by loss of IDH1\textsuperscript{mut} might contribute to the observed inhibition of chondrosarcoma formation in the xenograft model.

Angiogenesis is a key contributor to tumor progression and metastasis. HIF-1\(\alpha\) and VEGF are known to play crucial roles in the tumor angiogenic process [12, 25, 32]. It is established that VEGF expression is mediated by HIF-1\(\alpha\) during hypoxia. The VEGF gene contains a number of HIF-1\(\alpha\)-binding sites at its regulatory region, and HIF-1\(\alpha\) is able to activate the VEGF promoter [33, 34]. HIF-1\(\alpha\)-induced VEGF expression is implicated in the angiogenic switch in chondrosarcoma [32]. Interestingly, studies have shown that IDH mutation is associated with elevated levels of HIF-1\(\alpha\) and VEGF levels in IDH-mutant gliomas [18, 35]. Consistently, we found the expression of angiogenic markers, VEGFA and CD31, was significantly reduced in the IDH1\textsuperscript{mut} KO cell-derived tumors, suggesting that IDH1 mutation is associated with the angiogenic potential of chondrosarcoma.

**Figure 4:** HIF-1\(\alpha\) contributes to the tumorigenic function of IDH1 mutation in JJ012 cells. (a) Colony formation assay with JJ012 parental and IDH1\textsuperscript{mut} KO cells under hypoxic (1% O\(_2\)) and normoxic (21% O\(_2\)) conditions. 5 \times 10^3 cells per well were seeded in 6-well plates and incubated for 10–14 days. Graphs compare effects of hypoxia and normoxia on the number of colonies in the parental, KO1, and KO2 groups. Data indicate mean ± SEM of triplicate cultures and are representative of 3 independent experiments. *\(p < 0.05\). (b) Immunoblot shows HIF-1\(\alpha\) levels in JJ012 parental and IDH1\textsuperscript{mut} KO cells under normoxia (N) and hypoxia (H) conditions.
cells. This association was solidified by in vitro vascular tube formation assay, which shows that the secretome of IDH1mut KO cells substantially reduced HUVECs’ ability to form primitive vascular tubes in comparison with the secretome of parental cells. To be noted, our previous study also showed an association between IDH mutation and aberrant activation of integrin signaling in chondrosarcoma cells [22]. A few integrins have been implicated in blood vessel formation and regulation of cell growth, survival, and migration during tumor angiogenesis and metastasis [36, 37]. Interestingly, although VEGF expression is induced by HIF-1α during hypoxia [38], its expression can also be modulated by tumor integrins, resulting in efficient tumor angiogenesis under normoxic conditions [39]. Therefore, integrin and HIF-1α signaling might intertwine with each other by shared mediators such as angiogenic factors in the tumor microenvironment, and thus both pathways contribute to the process of angiogenesis in IDH-mutant chondrosarcomas [12, 36]. In any case, the established association in this study renders antiangiogenic molecules appealing candidates for combinatorial regimens with IDH1mut inhibitors for advanced chondrosarcomas. The use of angiogenesis inhibitors has been used as an adjunct to other forms of therapy for preventing development of malignant neoplasms [40]. Preclinical studies have shown the benefits of targeting angiogenesis in chondrosarcomas [41]. Given the modest results with IDH1mut inhibitors in chondrosarcoma [42], future efforts to improve the efficacy of these compounds might benefit from emphasis on biology-driven therapeutic strategies to improve response rates in IDH-mutated chondrosarcomas.

The present study was limited by the use of only one conventional chondrosarcoma cell line. In fact, due to the rareness of this malignancy, very few chondrosarcoma cell lines are available worldwide. Thus, more chondrosarcoma cell lines, or patients’ primary tumor cells, will be essential to further investigate and strengthen the concept explored in this study. Interestingly, we did not find a similar association between IDH mutation and HIF-1α in an IDH1-mutant fibrosarcoma cell line, HT1080, which was originally reported as a fibrosarcoma of bone, but is now considered to represent a dedifferentiated chondrosarcoma. We believe that the discrepancy is due to pathogenesis heterogeneity between the chondrosarcoma cell lines as conventional and dedifferentiated chondrosarcomas were shown to exhibit distinct biological behaviors and clinical characteristics. As described previously, differential biology was also observed within gliomas and leukemias with regards to the effects of IDH mutation on PHDs and HIF-1α activity [18–21]. Thus, much work remains to better understand the biology to fully clarify these discrepancies and identify the appropriate patient populations for specific targeted therapies. Nonetheless, herein, we identified a strong correlation between HIF-1α activation and IDH1 mutation status in chondrosarcoma cells. Furthermore, this study unravels one aspect of chondrosarcoma pathophysiology and provides insightful therapeutic possibilities such as combinatorial regimens of antiangiogenic agents with IDH1mut inhibitors for patients with advanced IDH mutated chondrosarcoma.

Data Availability

The experimental data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors have declared that no conflict of interest exists.

Authors’ Contributions

L.L., X.H., J.C.T., J.Y., and J.Y. conceived the experimental plan. X.H. and L.L. performed the experiments. All authors contributed to the data analysis. X.H., L.L., J.C.T., and J.E.E. wrote the manuscript. Xiaoyu Hu and Luyuan Li contributed equally to this work.

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