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The Krüppel-like zinc finger transcription factor, GLI-similar 1, is regulated by hypoxia-inducible factors *via* non-canonical mechanisms

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ABSTRACT

GLI-similar 1 (GLIS1) is important for the reprogramming of fibroblasts into induced pluripotent stem cells (iPSCs). However, the molecular mechanisms of regulation of *GLIS1* expression remain unclear. We have therefore examined *GLIS1* expression in various cancer cell lines and demonstrated that *GLIS1* expression was dramatically increased under hypoxic conditions. Importantly, *GLIS1* expression was significantly attenuated in VHL-overexpressing renal cell carcinoma cells compared to the VHL-deficient parent control. Moreover, promoter analysis demonstrated that *GLIS1* transcription was regulated by hypoxia through a hypoxia-inducible factors (HIFs)-dependent mechanism. Co-transfection experiments revealed that HIF-2α had greater potency on the *GLIS1* promoter activation than HIF-1α. Subsequent studies using wild-type and mutant HIF-2α demonstrated that DNA binding activity was not necessary but TADs were critical for *GLIS1* induction. Finally, co-transfection experiments indicated that HIF-2α cooperated with AP-1 family members in upregulating *GLIS1* transcription. These results suggest that the hypoxic signaling pathway may play a pivotal role in regulating the reprogramming factor *GLIS1*, *via* non-canonical mechanisms involving partner transcription factor rather than by direct HIF transactivation.

1. Introduction

The Krüppel-like protein Gli-similar 1 (*GLIS1*) was reported to be both temporally and spatially regulated, suggesting that it may play a role in the regulation of embryonic developmental programs at specific stages [1,2]. *GLIS1* expression was increased by phorbol-12-myristate-13-acetate (PMA) or interferon γ treatment and stable transfection of *GLIS*1ΔC that lacks activation domains prompted PMA-induced epidermal differentiation. This indicated a regulatory role for *GLIS1* in aberrant epidermal differentiation and remodeling of tumorigenic PMA-treated psoriatic skin [3]. Crucially, *GLIS1* markedly enhances the generation of induced pluripotent stem cells (iPSCs) from both mouse and human fibroblasts when it is expressed together with *OCT3/4* (*POU5F1*), *SOX2* and *KLF4*. *GLIS1* is able to replace oncogenic *MYC*, resulting in decreased tumorigenicity as well as improving safety and efficiency of iPS cell production, demonstrating its utility in stem cell biology [4].

Stem cells frequently reside in a specialized physiological microenvironment called the stem cell niche. The role of the niche is to maintain stem cell properties such as pluripotency and self-renewal. Recent evidence suggests that hypoxia may be an important physiological component of the microenvironment necessary for stem cell maintenance [5,6]. Consistent with this, hypoxic (low oxygen) conditions were able to improve the efficiency of iPS cell generation from mouse and human somatic cells [7]. Indeed, the master regulator of stemness Oct-4 is a direct transcriptional target of hypoxia-inducible factor-2-alpha (HIF-2α also known as endothelial PAS domain protein 1 or EPAS1) which is one of the main transcription factors of the hypoxia pathway further highlighting the importance of hypoxia in stem cells biology [8]. Mechanistically, hypoxia-inducible factor alpha (HIF-α) subunits are degraded through von Hippel–Lindau (VHL)-mediated ubiquitin–proteasomal degradation under normoxic conditions, and hypoxia stabilizes HIF-α thereby allowing their

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heterodimerization with a beta-subunit (HIF β/ARNT) that can bind to hypoxia response elements (HRE), resulting in the activation of multiple genes [9].

Therefore, given the importance of GLIS1, hypoxia and HIFs in stem cell biology, we examined the potential role of hypoxia in transcriptional regulation of *GLIS1*, and provide novel insights into the molecular mechanisms governing *GLIS1* expression under hypoxic conditions.

2. Materials and methods

2.1. Cell culture and RNA preparation

Human breast cancer (MCF-7, BT-474, MDA-MB-231, SKBR-3, and ZR-75-1), lung cancer (A549), liver cancer (HepG2), oral cancer (Ca9-22, KOSC-2, HSC-2, HSC-3 and HSC-4), renal cancer (RCC4/VHL, RCC4/pcDNA) and mouse liver cancer cell lines (Hepa1c4) were maintained in RPMI1640 or Dulbecco's modified Eagle's minimal essential medium (DMEM) (NACALAI TESQUE, Inc., Kyoto, Japan) containing 10% fetal bovine serum (FBS; BioWhittaker, Verviers, Belgium) as previously described [10–12]. For expression analysis, cells ($0.5–1.0 \times 10^6$/10 cm diameter dish) were cultured under normoxic (21% pO_2) or hypoxic (1% pO_2) conditions for 6, 12, 24, or 48 h in a hypoxia chamber. Cells were then harvested and stored at −80 °C until use. Total RNA was extracted from frozen cell pellets using the NucleoSpin® RNA II kit (MACHEREY–NAGEL, Düren, Germany) according to manufacturer instructions.

2.2. Real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis

Two micrograms of total RNA extracted from each cell line were reverse transcribed using the High-Capacity cDNA Archive™ Kit (Applied Biosystems, Foster City, CA, USA). A 1/200 dilution of the cDNA was subjected to real-time RT-PCR using primers (final concentration of 200 nM each) and MGB probe (final concentration of 100 nM, the Universal Probe Library: UPL, Roche Diagnostics, Tokyo, Japan) (shown in Supplementary Table) sets with Pre-Developed TaqMan™ Assay Reagents (Applied Biosystems) for quantitation of *GLIS1*, *HIF1A*, and *EPAS1* expression with *ACTB* as an internal housekeeping control. PCR reactions were carried out using 7500 Real-Time PCR System (Applied Biosystems) under the following standard conditions. Three independent measurements were averaged and relative gene expression levels were calculated as a ratio against *ACTB* expression for each cell line.

2.3. Immunoblot analysis

To analyze GLIS1, HIF-1α, or HIF-2α protein expression, whole cell extracts were prepared from cultured cells with or without hypoxic treatment as previously described [12,13]. Anti-GLIS1 (Epitomics, Burlingame, CA, USA), anti-HIF-1αs (BD Pharmingen, San Diego, CA, USA), anti-HIF-2α (Cell Signaling Technology, Inc., Danvers, MA, USA), or anti-β-actin (Sigma, St. Louis, MO, USA) were used as primary antibodies, and anti-mouse Ig or anti-rabbit Ig horseradish peroxidase conjugate (Amersham Life Science, Buckinghamshire, UK) in Can Get Signal® solution 2 were used as secondary antibodies. Immunocomplexes were visualized using the enhanced chemiluminescence reagent ECL Plus (NEN Life Science Products, Inc., Boston, MA, USA).

2.4. Plasmid constructs

A 1.62 kb DNA fragment including the 5′ untranslated region of the *GLIS1* gene (−1556 to +66 from the transcriptional start site at +1; GenBank: NT_032977) was amplified by PCR from MCF-7 genomic DNA and subcloned into the *Xho*I and *Bgl*II sites of the luciferase reporter plasmid pGL4.26 (Promega, Madison, WI, USA) and designated pGL4.26-GLIS1 Pro1556. Several 5′ deletion mutants of pGL4.26-GLIS1 Pro1556 were constructed by PCR cloning within the regions −732 to +66 (pGL4.26-GLIS1 Pro732) and −228 to +66 (pGL4.26-GLIS1 Pro228). Base change mutants of putative hypoxia response elements (HRE) or AP-1 binding sites, were generated by PCR site-directed mutagenesis as previously reported [12,13]. The HRE-luciferase reporter was constructed by subcloning of annealed HRE consensus oligos into the *Nhe*I and *Xho*I sites of pGL4.26 (pGL4.26-5xHRE). Full-length HIF-1α, HIF-2α mutants of HIF-2α 72–870 or HIF-2α 2–332 or MYC cDNA was amplified by PCR from HSC2 cDNA and subcloned into p3xFLAG-CMV™-10 (Sigma) or pcDNA™3.1/V5-His© (Invitrogen, Carlsbad, CA, USA) expression vectors. All constructs were confirmed by sequence analysis. ARNT (pCMV-ARNT) and IPAS (pCMV-FLAG-IPAS) expression vectors are as previously described [14]. Rat Jun/human β-actin and human FOS expression vectors were generously provided by Dr. Masaharu Sakai (Hokkaido University) [15].

2.5. Luciferase reporter assays

Transient transfections were performed with combinations of reporter constructs (0.2 μg/15 mm well) and expression vectors (0.1–0.4 μg/15 mm well) mixed with 0.9 μl of TransIT®-LT1 Transfection Reagent (TaKaRa Bio, Inc., Shiga, Japan). The Renilla luciferase vector (pRL-SV40, 1.0 ng/15 mm well, Promega) was used as a transfection efficiency control. Cells were incubated for 24 h after transfection prior to analysis of luciferase reporter activity. Luciferase luminescence was measured using a single-sample luminometer, the Biolumat LB 9505 (BERTHOLD TECHNOLOGIES GmbH & Co. KG, Bad Wildbad, Germany) with the Dual-Luciferase® Reporter Assay System (Promega). Promoter activity was calculated as the ratio of firefly to Renilla luciferase readings, and the average of at least three independent experiments was taken.

2.6. Statistical analysis

All statistical tests were performed using the StatView® version 5.0 software (SAS Institute Inc., NC, USA) and Microsoft® Excel® 2008 for Mac version 12.3.6. Student's *t*-test was used to determine *P*-values with * indicating $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

3. Results

3.1. Hypoxia induces GLIS1 expression in cancer cells in a VHL- and HIF-dependent manner

To determine how *GLIS1* was transcriptionally regulated, we first examined expression levels of *GLIS1* in cancer cell lines of diverse origins, including head and neck, breast, lung, liver and renal cancers under hypoxic compared to normoxic conditions. Real-time RT-PCR analysis revealed that baseline expression levels of *GLIS1* were variable among cell lines with MCF-7 breast and RCC4 renal cancer cells showing the highest expression. Notably, *GLIS1* expression was significantly increased under hypoxic conditions in 6 cell lines (breast cancer MCF-7, BT-474, lung cancer A549, liver cancer HepG2, oral cancer Ca9-22 and renal cancer RCC4, Fig. 1A–E) suggesting that this was a common response to hypoxia. Importantly, *GLIS1* expression was significantly attenuated in RCC4 cells transfected with VHL expression vector (RCC4/VHL) compared to the original VHL-deficient RCC4 parental cell line that constitutively expresses the HIFs. This suggested a role for the VHL-mediated degradation of HIFs on *GLIS1* regulation,

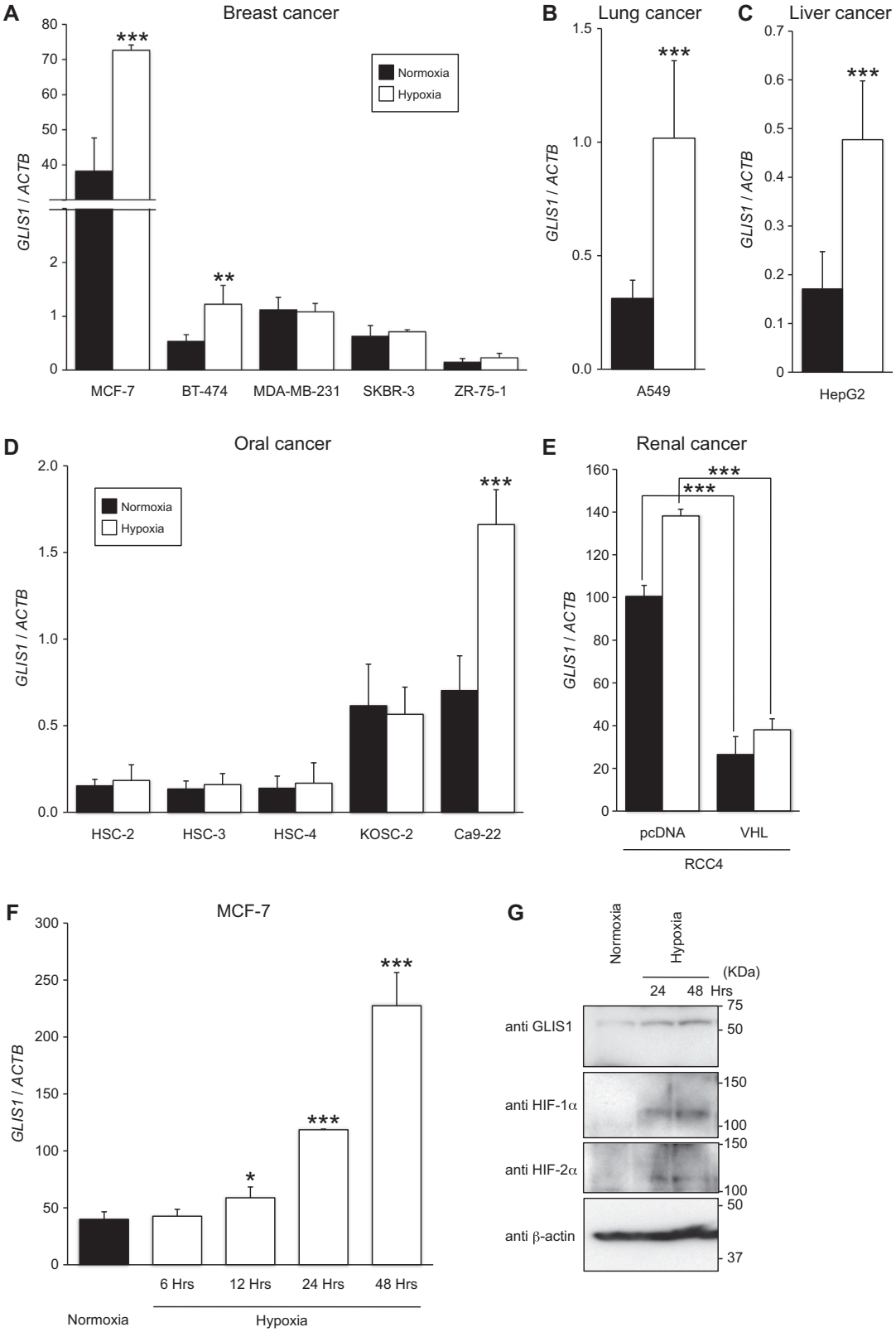

Fig. 1. *GLIS1* is expressed in diverse cancer cell lines under normoxic and hypoxic conditions. Expression levels of *GLIS1* in various cancer cell lines of different tissue origins, including (A) breast, (B) lung, (C) liver, (D) oral, and (E) renal cancers, were quantified by real-time RT-PCR following normoxia or hypoxia treatments for 24 h. Time-course kinetics of *GLIS1* expression were obtained after hypoxia treatment of MCF-7 cells for the indicated durations followed by analysis using real-time RT-PCR (F) or immunoblotting (G). Relative *GLIS1* expression levels were calculated after normalization against housekeeping *ACTB* expression. Each bar represents the mean + SD for at least three independent experiments. Statistical significance was calculated between normoxic and hypoxic samples, or for the indicated paired samples with $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

supported by knock-down experiments using specific siRNAs against *HIF1A* (siHIF1A) and *EPAS1*/*HIF2A* (siEPAS1) (Supplementary Fig. 2). Time-course kinetics of *GLIS1* expression showed that the gene was induced beginning after 12 h incubation under hypoxic conditions, and continued to increase robustly up to the 48 h time point in our experiments (Fig. 1F). Furthermore, immunoblot analysis showed increased expression of the GLIS1 protein under hypoxic conditions consistent with the RT-PCR analysis (Fig. 1G). Interestingly while HIF-1α protein levels were transiently and significantly accumulated after 24 h hypoxia, HIF-2α protein remained weakly expressed in MCF-7 cells.

3.2. Hypoxia regulates GLIS1 promoter activity via HIF-signaling but not through the HRE consensus

To clarify the mechanisms by which HIFs mediate the induction of *GLIS1*, we next subcloned the 5′ flanking region of exon 1 and promoter region of *GLIS1* (−1556 to +66 with the transcriptional start site at +1) containing two HRE consensus sequences into the luciferase reporter vector pGL4.26 (pGL4.26-GLIS1 Pro1556) (Fig. 2A and C, and Supplementary Fig. 1). Transient transfection experiments and luciferase assays indicated that the cloned *GLIS1* 5′ fragment had strong promoter activity under normoxic conditions in both MCF-7 and MDA-MB-231 cells compared to the empty control reporter pGL4.26 (Fig. 2B and C). Furthermore, promoter activity was significantly increased under hypoxic conditions in both cell lines. To identify the *GLIS1* promoter elements important for this mechanism, several mutants were constructed (Fig. 2D and C, and Supplementary Fig. 1). Deletion mutants lacking one or both of the HRE still produced significant induction under hypoxic conditions. Furthermore, point mutations in either of the consensus HREs did not affect hypoxic induction of reporter activity. Taken together, these results show that the hypoxia responsive region of the *GLIS1* promoter is contained within the shortest fragment from −228 to +66 of *GLIS1* (Fig. 2D). Since there were no consensus HRE sequences found in this short fragment, we focused on the similar GTG-motifs that are adjacent to the transcriptional start site (Fig. 2E, and Supplementary Fig. 1). Point mutations in the different GTG-motifs (mt3 or mt4) to GAG resulted in significant reduction of *GLIS1* promoter activity but did not affect hypoxic induction (Fig. 2E). Interestingly, point mutation in the most 5′ GTG-motifs (mt5) abolished basal activity of the *GLIS1* promoter and also hypoxic inductions. Therefore this site may correspond to an important regulatory element necessary for the expression of *GLIS1*.

3.3. HIF-2α predominantly regulates GLIS1 promoter activity via its C-terminal domain

To determine the functional domains of HIF that drive *GLIS1* transcriptional activity, a series of co-transfection experiments were performed in MCF-7 cells. Notably, FLAG-tagged HIF-2α dramatically transactivated the promoter, although both HIF-1α equally activated the consensus HRE-driven luciferase reporter in

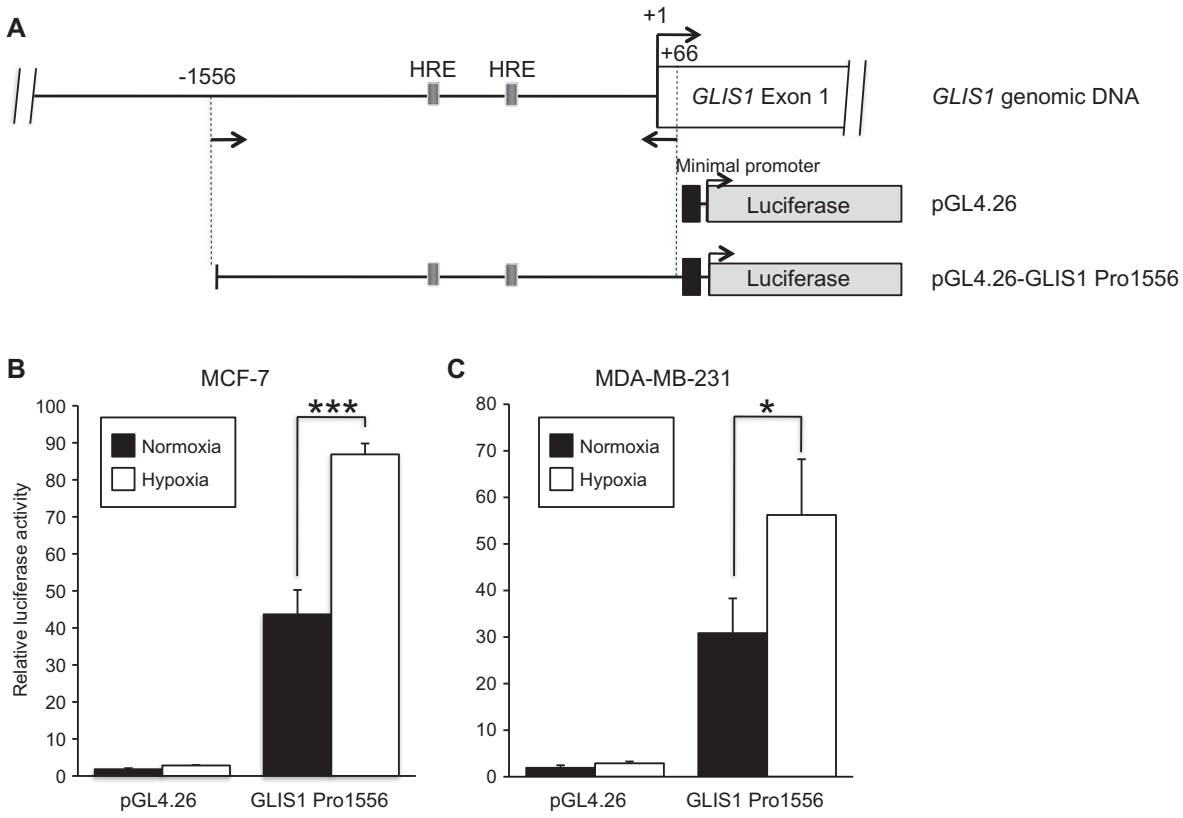

Fig. 2. Hypoxia activates the immediate upstream promoter region of *GLIS1* independently of the hypoxia response elements (HREs). The 5′ promoter region (−1556 to +66 from the transcriptional start site at +1) of *GLIS1* was subcloned into pGL4.26 to generate the pGL4.26-GLIS1 Pro1556 luciferase reporter construct (A). The positions of the consensus hypoxia response elements (HRE) on the *GLIS1* promoter are indicated. The activity of pGL4.26-GLIS1 Pro1556 was evaluated by transient transfection luciferase assays in MCF-7 (B) or MDA-MB-231 (C) cells under normoxic and hypoxic conditions. A series of deletion or HRE mutants for the pGL4.26-GLIS1 Pro1556 construct (D), or GTG-motif mutants of the pGL4.26-GLIS1 Pro228 deletion construct (E) were also transiently transfected and luciferase activity was evaluated as before. Relative firefly luciferase activity was calculated as a ratio against renilla activity produced by the pRL-SV40 transfection efficiency control. Each bar represents mean + SD for at least three independent experiments. Statistical significance was calculated between normoxic and hypoxic conditions with $^{*}P < 0.05$ and $^{***}P < 0.001$.

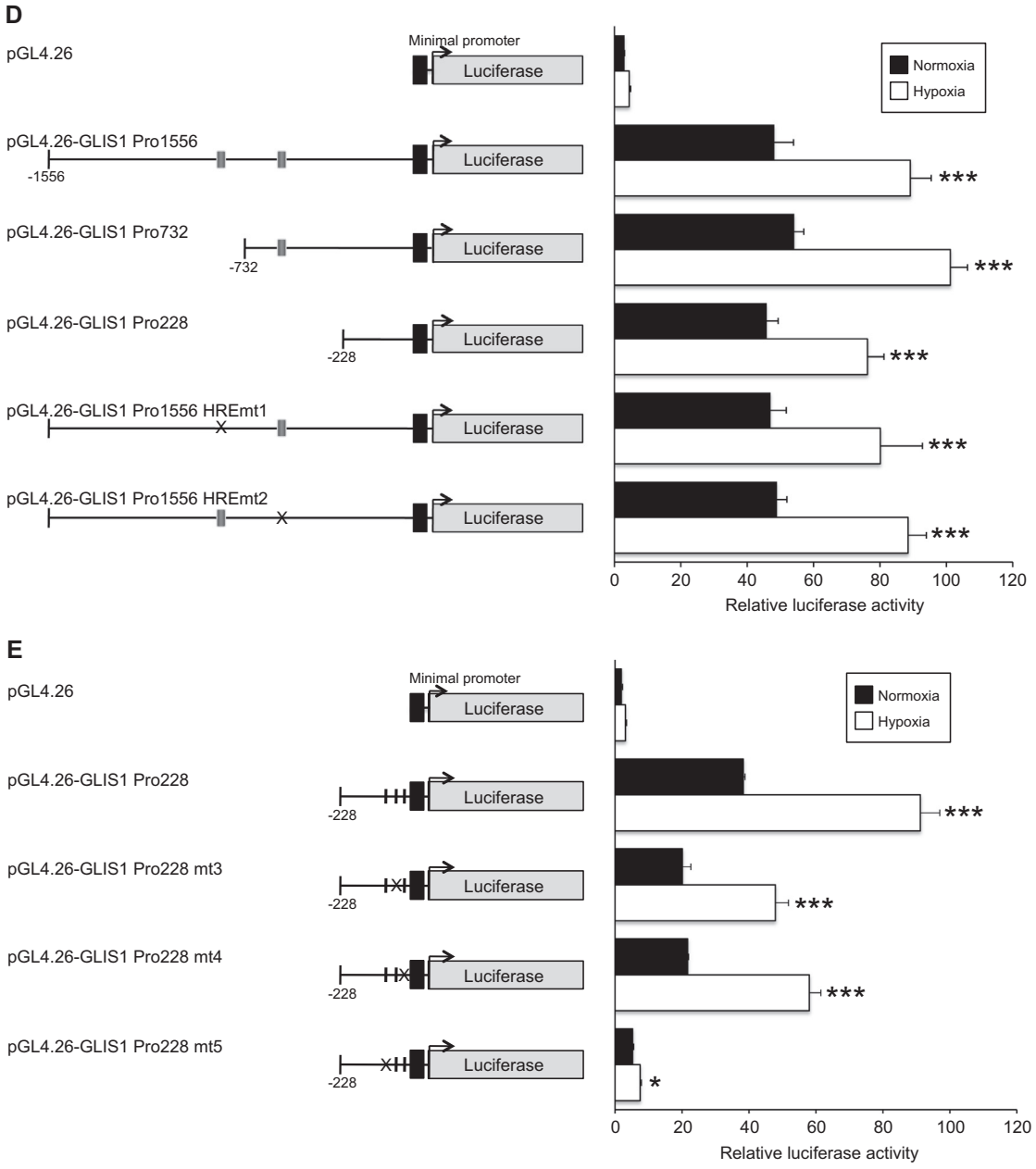

Fig. 2 (*continued*)

identical conditions (Fig. 3A and B). We thus sought to find the HIF-2α specific protein domains that were responsible for transcriptional activation. We performed further co-transfection experiments with mutated HIF-2α (Fig. 3C). Surprisingly, a mutant HIF-2α lacking the basic-helix-loop-helix (bHLH) DNA binding domain could activate *GLIS1* promoter, although this mutant protein was not able to bind DNA (Fig. 3D). On the other hand, a mutant HIF-2α lacking the C-terminal transactivation domains (TADs) failed to activate the promoter. Furthermore, coexpression of the inhibitory PAS domain protein (IPAS) interfered with HIF-2α-activated *GLIS1* promoter activity, suggesting a possible role for protein–protein interaction that may include dimerization with ARNT, a classical transcriptional co-partner of HIFs (Fig. 3E). However, co-transfection experiments using the Arnt-deficient hepatoma mouse cell line Hepa1c4 suggested that Arnt was inadequate for the activation of the *GLIS1* promoter (Supplementary Fig. 3). Therefore, HIF-2α transactivation of the *GLIS1* promoter may not depend on ARNT but require interaction with alternative transcriptional partners.

3.4. HIF-2α cooperates with AP-1 transcription factor family members to drive GLIS1 expression

To determine what alternative factors and regulatory mechanisms may be acting upon the *GLIS1* promoter, we interrogated transcription factor chromatin immunoprecipitation-sequencing (ChIP-Seq) datasets from the ENCODE consortium to identify candidate transcription factor binding within the immediate 5′ promoter region of *GLIS1*. As a result, we found evidence for the binding of AP-1 family members in diverse cell types within the vicinity of the *GLIS1* transcription start site (Supplementary Fig. 4). Based on these results we evaluated the effects of AP-1 family members, Jun and FOS as well as the proto-oncogene MYC on the E-box motif GTG on *GLIS1* promoter activities in MCF-7 cells.

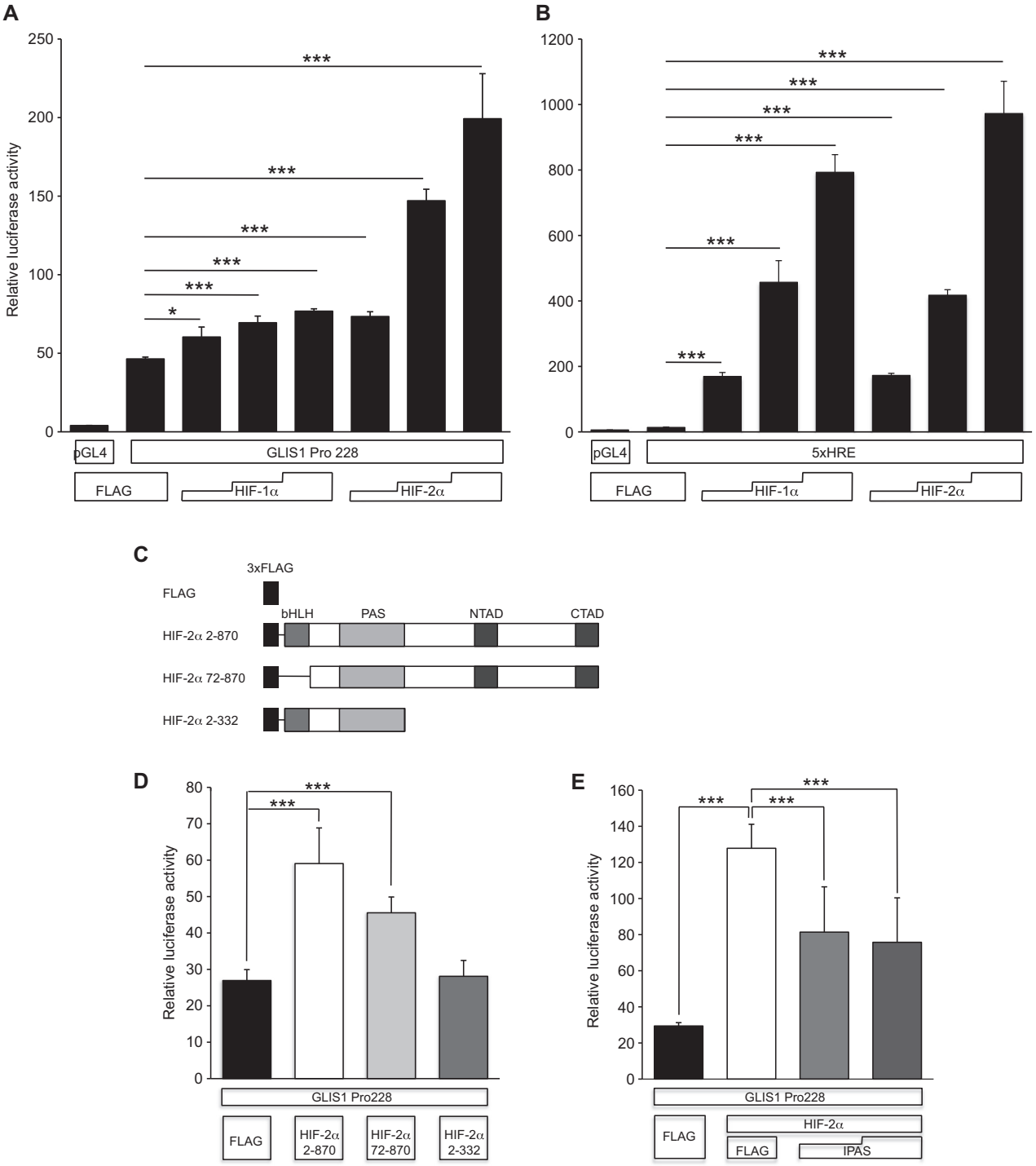

Fig. 3. HIF-2α predominantly regulates *GLIS1* promoter activity by non-canonical mechanisms. Increasing amounts of FLAG-tagged HIF-1α or HIF-2α expression vectors were co-transfected with the pGL4.26-GLIS1 Pro228 promoter luciferase construct (A) or HRE-driven luciferase reporter (B) in MCF-7 cells, and incubated under normoxic conditions for 24 h. Schematic representation of FLAG-tagged full-length (2–870 residues) and deletion mutants (72–870 or 2–332 residues) of HIF-2α are shown (C). The various FLAG-tagged HIF-2α expression vectors (D) or full-length HIF-2α with or without IPAS (E) were co-transfected with the minimal pGL4.26-GLIS1 Pro228 promoter luciferase reporter in MCF-7 cells, and incubated under normoxic conditions for 24 h. Relative firefly luciferase activity was calculated as above. Each bar represents mean + SD for at least three independent experiments. Statistical significance was calculated for all test conditions over the empty vector control (FLAG) and between HIF-1α, HIF-2α or others with $^{*}P < 0.05$ and $^{***}P < 0.001$.

Transient transfection experiments showed that overexpression of Jun, FOS, or MYC alone could transactivate the promoter where Jun showed the strongest effect (Fig. 4). Interestingly, co-transfection of HIF-2α with Jun or FOS, but not MYC, synergistically increased *GLIS1* promoter activity, suggesting cooperation among these factors.

4. Discussion

A novel Krüppel-like protein Gli-similar 1 (*GLIS1*) was recently shown to be a reprogramming factor that can be used to replace *MYC* during the production of iPSCs together with *POU5F1*, *SOX2* and *KLF4*, although the molecular mechanisms governing *GLIS1*

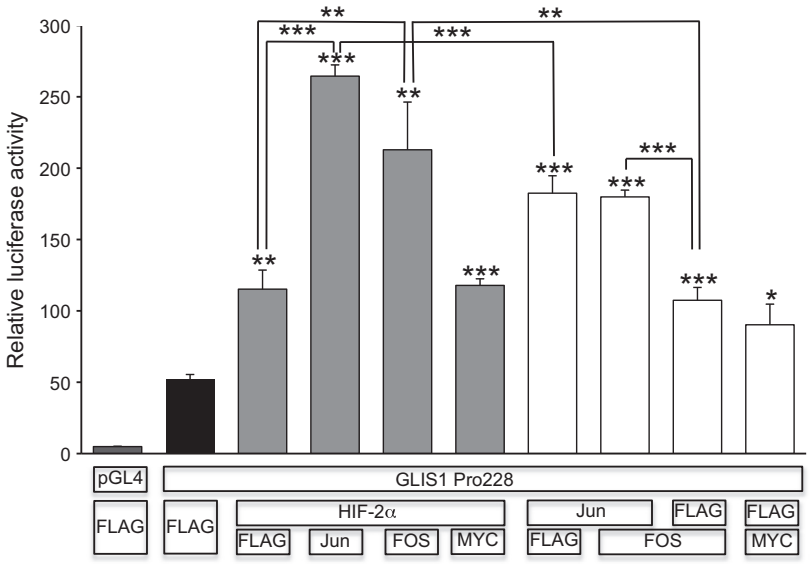

Fig. 4. HIF-2α regulates *GLIS1* promoter activity by possible cooperation with AP-1 family members. The *GLIS1* promoter luciferase reporter (pGL4.26-GLIS1 Pro228) was co-transfected with or without the transcription factors HIF-2α, Jun, FOS, MYC or various permutations as indicated in MCF-7 cells, and incubated under normoxic conditions for 24 h. Relative firefly luciferase activities were calculated as above. Each bar represents mean + SD for at least three independent experiments. Statistical significance was calculated between empty vector control (FLAG) and HIF-2α overexpression samples, empty vector (FLAG) and other samples, or paired as indicated with $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

function were not determined [3]. Here, we demonstrated for the first time that hypoxia, a prevalent and important microenvironment condition for the maintenance of stemness, regulated *GLIS1* expression *via* novel mechanisms. Hypoxia-inducible factors, especially HIF-2α, were found to cooperate with AP-1 family members in upregulating *GLIS1* transcription under hypoxic conditions.

When we evaluated expression levels of *GLIS1* in diverse types of cancer cell lines, we found that *GLIS1* expression was variable among cell lines with relatively high expression found in MCF-7 breast and RCC4 renal cancer cells, but with low levels in most of other cells. Expression of *GLIS1* in cancer cells has not been reported so far, but the gene is known to be temporally and spatially expressed in a dynamic manner during mouse embryonic development that is consistent with the specification of downstream cell fates from stem and progenitor cells [1,2]. Because it might have specific tissue and/or stage-dependent expression, further analyses are obviously needed to understand the *GLIS1* expression pattern in human normal and cancer cells.

Interestingly, we found increased expression of *GLIS1* under hypoxic conditions in a number of cancer cell lines. Furthermore, *GLIS1* expression was significantly lowered in RCC4 cells transfected with the VHL expression vector (RCC4/VHL) compared to the VHL-deficient parent. Parental RCC4 cells constitutively stabilize HIFs even in normoxic conditions due to loss of VHL, but restoration of VHL by transfection results in the rapid degradation of HIFs. Taken together, these observations suggest a role for the VHL–HIF mechanism on the regulation of *GLIS1* expression. We indeed found that suppression of HIFs attenuated hypoxic induction of *GLIS1*, suggesting that hypoxia-driven HIFs transcriptionally upregulate *GLIS1*. The time-course experiments showing that *GLIS1* begins to increase under hypoxic conditions after 12 h incubation or more, suggesting that the gene was subjected to more immediate transcriptional regulation rather than inhibition of mRNA degradation further downstream.

We next subcloned the 5′ region of *GLIS1* into luciferase reporter vectors and performed transient transfection experiments to clarify the molecular mechanisms of transcriptional regulation by hypoxia. Our results showed strong promoter activity of the cloned *GLIS1* fragment under normoxic conditions, and this was significantly increased under hypoxia in both MCF-7 and MDA-MB-231 cells, suggesting the presence of critical regulatory elements in the promoter region. We indeed found two consensus HRE sequences on the *GLIS1* promoter, but a series of transient transfection experiments surprisingly indicated that the HRE mutations did not affect basal promoter activity and hypoxic inductions, suggesting adjacent promoter regions may instead be responsible. Therefore, we searched for similar sequences to the HREs namely the GTG-motifs that commonly allow binding of basic helix-loop-helix (bHLH)-Per/Arnt/Sim (PAS) family members [16]. Strikingly, one of the GTG-motif mutants disrupted both *GLIS1* basal activity and hypoxic response, suggesting that this site was critical for transcriptional regulation under both normoxic (basal) and hypoxic conditions. Interestingly, we noticed that this GTG-motif overlaps with an AP-1 binding sequence. ENCODE data analyses of ChIP-seq datasets revealed that there were binding of AP-1 family members in the region adjacent to the *GLIS1* transcriptional start site in different cell types. Our reporter experiments confirmed that the *GLIS1* promoter was indeed activated during co-transfection with the AP-1 family members, JUN or FOS. These results provide additional insights together with a previous report showing that the mRNA levels of *Glis1* were significantly induced in mouse skin upon treatment with PMA [2].

Moreover, deletion mutants of HIF-2α indicated that its DNA binding domain may not be necessary to activate *GLIS1* promoter, but highlighted that it may cooperate with other proteins through its C-terminal transactivation domains. Because the deletion of the bHLH domain of HIF-2α abolishes canonical transcriptional activity on HRE-reporters, our result suggests non-canonical mechanisms at work on the *GLIS1* promoter. It is known that the bHLH domain of HIF-2α participates not only in DNA binding activity but it is also important for dimerization with ARNT pointing towards a role for the transcriptional co-partner in the mechanism [17]. In fact, co-transfection experiments using the Arnt-deficient hepatoma mouse cell line Hepa1c4 suggested that Arnt might be necessary but inadequate for the activation of the *GLIS1* promoter (Supplementary Fig. 3). This led to the search for alternative candidate

factors besides Arnt and demonstrated for the first time that HIF-2α may cooperate with AP-1 family members. Our co-transfection experiments showed that JUN seemed to function in a more potent manner than FOS on the *GLIS1* promoter. While there have been previous reports linking HIF-1α interaction with JUN, and cooperativity with AP-1 binding sites under hypoxic conditions [18,19], whether physical interaction between HIF-2α and JUN occurs remains unclear but this could account for the functional cooperation between the 2 transcription factors in *GLIS1* regulation. It will be of interest to elucidate the molecular mechanisms behind these novel cooperative associations between HIF-2α and AP-1 given the importance of both factors in cancer disease.

In conclusion, we demonstrated for the first time that hypoxia-regulated *GLIS1* expression occurs *via* a novel mechanism requiring the cooperation between HIFs, notably HIF-2α, and AP-1 family members. This highlights possible roles for the interplay between these factors in reprogramming, dedifferentiation and/or maintenance of stemness in normal versus cancer cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.10.083.

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