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A novel hypoxic lncRNA, HRL-SC, promotes the proliferation and migration of human dental pulp stem cells through the PI3K/AKT signaling pathway

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Abstract

Background: Human dental pulp stem cells (hDPSCs) are critical for pulp generation. hDPSCs proliferate faster under hypoxia, but the mechanism by which long noncoding RNA (lncRNA) regulates this process is not fully understood.

Methods: Novel lncRNAs were obtained by reanalysis of transcriptome datasets from RNA-Seq under hypoxia compared with normoxia, and a differential expression analysis of target genes was performed. Bioinformatics analyses, including gene ontology analysis, Kyoto Encyclopedia of Genes and Genomes pathway analysis and gene set enrichment analysis, were used to understand the function of key novel lncRNAs. hDPSCs were isolated from dental pulp tissue. EdU and scratch wound healing assays were used to detect the proliferation and migration of hDPSCs. qRT-PCR was used to detect changes in the RNA expression of selected genes. RNA fluorescence in situ hybridization, small interfering RNA, qRT-PCR and Western blot analysis were used to explore the function of key novel lncRNAs.

Results: We identified 496 novel lncRNAs in hDPSCs under hypoxia, including 45 differentially expressed novel lncRNAs. Of these, we focused on a key novel lncRNA, which we designated HRL-SC (hypoxia-responsive lncRNA in stem cells). Functional annotation revealed that HRL-SC was associated with hypoxic conditions and the PI3K/AKT signaling pathway. HRL-SC was mainly located in the cytoplasm of hDPSCs and had stable high expression under hypoxia. Knockdown of HRL-SC inhibited the proliferation and migration of hDPSCs and the expression levels of PI3K/AKT-related marker proteins. Furthermore, the AKT activator SC79 partially offset the inhibitory effect caused by the knockdown, indicating that HRL-SC promoted hDPSCs through the PI3K/AKT signaling pathway.

Conclusions: Hypoxia-responsive lncRNA HRL-SC promotes the proliferation and migration of hDPSCs through the PI3K/AKT signaling pathway, and this understanding may facilitate the regenerative application of hDPSCs.

Keywords: Human dental pulp stem cells, Hypoxia, lncRNA, Proliferation and migration

Introduction

Human dental pulp stem cells (hDPSCs) are located in dental pulp tissue and have a high capacity for self-renewal and multidirectional differentiation [1]. Dental pulp tissue can be obtained from discarded human teeth, including wisdom teeth or premolars extracted for orthodontic reasons [2]. The advantages of hDPSCs include little ethical controversy, low immunogenicity and strong

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Zeng *et al. Stem Cell Research & Therapy*   (2022) 13:286

Page 2 of 16

proliferation ability compared with bone marrow mesenchymal stem cells (BMSCs) [3, 4]. In addition, hDPSCs can differentiate into primary odontoblasts and pulp cells to form pulp-like tissues. Thus, hDPSCs are ideal seed cells for dental pulp tissue engineering and have been widely used in pulp regeneration research [5, 6].

R. Schofield first proposed the concept of stem cell niches, which refers to a specific microenvironment that regulates the fate of stem cells [7]. Stem cells in vivo reside in a hypoxic microenvironment, which is one of the most important stem cell niches that maintains stem cell characteristics [8]. The oxygen concentration of different hypoxic microenvironments depends on the organ blood flow; thus, less irrigated organs receive less oxygen, ranging from 1 to 14% [9, 10]. The oxygen concentration of mesenchymal stem cells in bone marrow has been reported to be 1–7% [11]. Dental pulp tissues are surrounded by rigid dentin, and the apical foramen is the only vascular access, indicating that the pulp tissues are also at lower oxygen tension levels [12]. It has been reported that the oxygen concentration of rat incisor pulp is approximately 3% [12]. In general, hDPSCs are cultured in 21% oxygen under in vitro conditions, and high oxygen tension increases oxidative stress, resulting in damage to cell metabolism [13]. Therefore, hypoxic culture provides a better simulation of the physiological conditions that are necessary for stem cell culture [14]. Hypoxia enhances the proliferation and migration of hDPSCs [15–17]. In addition, increased expression of pluripotency markers in hDPSCs such as SRY-box transcription factor 2 (SOX2), octamer-binding protein 4 (Oct-4) and c-Myc were observed under hypoxia [18]. The hypoxic signaling response is orchestrated by hypoxia inducible factor (HIF), which activates glycolytic enzymes to provide energy for stem cells [19, 20]. Furthermore, because hypoxia maintains an undifferentiated state and promotes the proliferation of stem cells, it is necessary to explore the mechanisms of stem cell behavior under hypoxia.

lncRNAs are a class of RNA molecules of more than 200 nt without protein coding potential [21]. lncRNAs are involved in stem cell regulation at the transcriptional, posttranscriptional and epigenetic levels, interacting with molecules to act as guides, scaffolds, decoys and tethers to regulate cell proliferation, migration, apoptosis and differentiation [22–24]. There is a group of lncRNAs that is specifically expressed under hypoxia, called hypoxia-responsive lncRNAs (HRLs) [25, 26]. Increasing evidence indicates that lncRNAs participate in the hypoxia-related cellular processes of stem cells, including proliferation, migration, differentiation and stemness. Some studies have demonstrated that lncRNAs that are modulated by hypoxia promote the proliferative and migratory capacity of mesenchymal stem cells [27–29]. For example, lncRNA-miRNA−mRNA network analysis with lncRNAs as the core components was performed to understand the molecular expression patterns of hypoxia-promoting effects in olfactory mucosa mesenchymal stem cells [27]. It was also revealed that the hypoxia-responsive lncRNA STL may be the key component of a coding-noncoding gene coexpression network that regulates the functions of stem cells [30].

However, very few studies have explored the roles of lncRNAs in the regulation of hDPSCs, and the mechanisms involved remain largely unclear. Here, we reanalyzed our previous RNA-seq data of hDPSCs under hypoxia and normoxia, and performed a differential expression analysis of target genes to determine the functions of novel lncRNAs. We identified a key novel lncRNA, HRL-SC, which was significantly upregulated in hDPSCs under hypoxia compared with normoxia. Functional annotations revealed that HRL-SCs were closely associated with hypoxia signatures. Knockdown of HRL-SC inhibited the proliferation and migration of hDPSCs under hypoxia and decreased the activity of the PI3K/AKT signaling pathway. However, the activators of AKT offset the inhibition caused by knockdown of HRL-SC. Our data show that hypoxia-responsive HRL-SC promotes the proliferation and migration of hDPSCs via the PI3K/AKT signaling pathway and provides a potential molecular mechanism of hDPSCs under hypoxia.

Materials and methods
RNA-Seq data processing
RNA-Seq had been performed for hDPSCs under normoxia and hypoxia in our previous study, which can be obtained from NCBI GEO: GSE118046. Fastqc (version 0.11.9) was used to access the sequencing quality and Trimmomatic was used to filter low-quality sequencing reads. After the quality control, reads were aligned to the reference genome (Hg19) using HISAT2 (version 2.1.0) and the sam files for each sample were obtained. Next, sam files were converted to sorted bam files using Samtools (version 1.13). Assembly of bam files and gene quantification (FPKM values) were using StringTie (version 2.1.5) and the '-merge' function was performed to merge the transcript.

Identification of novel lncRNA
The pipeline for identifying novel lncRNA from assembled transcript are as follows: (1) filter transcripts with class code 'i, j, u, x' from Gffcompare of String-Tie; (2) remove the transcripts that matched annotations of known lncRNA; (3) filter transcripts with length ≥ 200 bp, exon numbers ≥ 1; (4) filter transcripts without coding potential using the protein-coding gene

Zeng *et al. Stem Cell Research & Therapy*   (2022) 13:286

Page 3 of 16

prediction tools including Coding-Non-Coding-Index (CNCI, version 2), Coding Potential Calculator (CPC, version 2), Coding Potential Assessment Tool (CPAT) and Pfam-scan (version 14.3) [31]. Finally, retaining transcripts with FPKM > 0 in at least one sample were novel lncRNAs.

Differential expression analysis

Differential expression analysis was used to identify the key novel lncRNAs under hypoxia. Expression matrix of FPKM values for mRNA and novel lncRNA was imported into R (version 3.63) and were analyzed using Linear Models for Microarray data (limma, https://bioconductor.org/packages/limma/) package in Bioconductor. Up- or downregulated differentially expressed mRNA/novel lncRNAs between samples under hypoxia and normoxia were identified with the cutoff criteria of $P < 0.05$ and |fold change (FC)| > 2.

LncRNA target prediction and functional annotation analysis

To understand the function of key lncRNA, LncTar (version) was used to explore the potential interaction between key lncRNA and differentially expressed genes. LncTar is a bioinformatics tool that calculates the free energy between lncRNA and target genes and a threshold of -0.1 ndG (default parameter) was set. Next, the target genes were imported into Database for Annotation Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) to perform GO enrichment and KEGG pathway analysis. To further explore the function of lncRNA HRL-SC, GSEA (version 4.1.0) was performed using c2.all.v7.4.symbols.gmt (http://software.broadinstitute.org/gsea/downloads.jsp) as a reference gene set. $P < 0.05$ and FDR < 0.25 were considered statistically significant.

Cell culture and identification

The hDPSCs were obtained from the third molar of patients aged 18–25 years indicated for extraction at the Department of Stomatology in Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, China. All experimental protocols were approved by the Ethical Committee of Southern Medical University. The isolated hDPSCs were cultured in Dulbecco modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; ExCell Bio, Shanghai, China), 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma, St. Louis, Mo, USA) at 37 °C in humidified atmosphere containing 5% CO_2 with different oxygen concentrations. Induced conditioned medium was used for cell induction and differentiation. The medium was replaced with the medium containing 5 μg/mL of SC79 (10 μM, GlpBio) in AKT

activation experiments. The culture media were renewed every 3 days.

After 21 days of differentiation, Alizarin Red and ALP activity staining were performed to verify the formation of mineral nodes and purple-colored precipitate. Besides, the cells were incubated for surface markers including CD45, CD34, CD90, CD44 and CD29 and then were detected by flow cytometry to identify the cell phenotypes of hDPSCs.

Cell transfection

The siRNAs against lncRNA and control siRNA were obtained from GeneChem (Shanghai, China). The details of sequences could be seen in Table 1. The siRNAs and control siRNA were transfected using riboFECT™ CP (Ribobio, Guangzhou, China) according to the manufacturer's protocol.

RNA-fluorescent in situ hybridization (RNA-FISH)

The subcellular localization of HRL-SC was detected by RNA-FISH kit (GenePharma, China) according to manufacturer's protocol. Briefly, the treated hDPSCs were washed with PBS and fixed with 4% Paraformaldehyde for 15 min. Then, the cells were permeabilized with 0.5% Triton X-100 in PBS hybridization buffer and washed with SSC solution. Cy3-labeled HRL-SC probes was used to perform hybridization in the dark overnight. After being washed with 0.1% Tween 20 and SSC solution, the cells then were observed and photographed under an inverted fluorescent microscope.

Scratch wound healing assay

The migration capability of hDPSCs was characterized by scratch wound healing assay. Cells were cultured with DMEM supplement with 10% FBS in 6-well plates until they reached confluence. After starving for 24 h with serum-free DMEM, scratches were generated with a 1-mL pipette tip in each well. After scratching for 0 h (right after scratch), 12 h and 24 h, the migration of cells was observed and photographed under an inverted microscope. The scratch wound healing area across each scratch was analyzed calculated by using ImageJ software.

Cell proliferation assay

The proliferation capability of hDPSCs was measured using the EdU (meilunbio, Dalian, China) assay. The hDPSCs were labeled with EdU for 2 h and then fixed, washed, permeabilized and stained according to the manufacturer's protocol. After cells were washed, the DNA was stained with Hoechst 33342; then, each well was observed and photographed under an inverted fluorescent microscope. The quantification was performed using with analyze particles function of ImageJ software

Zeng *et al. Stem Cell Research & Therapy*   (2022) 13:286

Page 4 of 16

Table 1 Primers for the qRT-PCR and probes used in cell transfection

Gene name	sense (5′–3′)	Anti-sense(5′–3′)
β-actin	TCACCATGGATGATGATATCGC	ATAGGAATCCTTCTGACCCATGC
MSTRG.20649.1	TGGAGGGAAGACACTGTCCTAT	TGCTTGGTGGGGGTTATCCA
MSTRG.8144.1	GGATTGTTCCCCACAAACCA	ATTCCAGTAACCTGCCCTTGG
MSTRG.27918.3	TCAAAGTGGGTCAGTGCAGG	CCTCAGGCCAACAGAGTAGC
MSTRG.31275.1	TCTCACATCCAATTCCCCGT	GAGTGCCACCTAGTGCTACC
MSTRG.16037.1	GAAAAGGGGTGTCAGCAACAA	TCACACAGACATCACTCCGTC
MSTRG.6909.1	AGCTGCAAAAGGCATCCTAGT	CCCCACAGCGTTTAATGGAAG
MSTRG.11228.1	TCGGGTGATGATGGTTCCAC	GAACCACCTCCCGAACCCAA
MSTRG.10485.1	CGGTGGCTCACTCATTGACA	TGGACTTGGTCTGTTAAGGGC
MSTRG.15583.1	TGCCACATCGTGTGTTCTGT	AGACACGGTAACCACACACC
MSTRG.11101.4	CAGACGCTCCCCGCAGA	ATCAAGACACCTGCCTTCCA
PDK1	CTGTGATACGGATCAGAAACCG	TCCACCAAACAATAAAGAGTGCT
siRNA1	CCACAAAUGGCCUGAACUGUAUU	UACAGUUCAGGCCAUUUGUGGUU
siRNA2	CCUCAGGUAGCUGCUGUUUUGUTT	ACAAACAGCAGCUACCUGAGGTT
siRNA3	UGCUGUUUGUGUGAGAAAGAATT	UUCUUUCUCACACAAACAGCATT

to count the number of positive cells. The proliferation rate was defined as the ratio of EdU-positive cells to total Hoechst-positive cells from at least three random fields of view.

RNA extraction and RT-PCR

Total RNA was extracted from each group using EZ-press RNA Purification Kit (EZBiosicence, USA) according to the manufacturer's protocol and quantified by NanoDrop ND-1000 (Thermo Scientific, USA).

Reverse transcription was performed using Color Reverse Transcription Kit (EZBiosicence, USA) with gDNA remover. A volume of 13 μL RNA solution of each sample (containing 1 μg RNA) was treated with 2 μL provided gDNA remover to remove genomic DNA. Each reaction was set for 20 μL, including 13 μL processed RNA solution and 5 μL 4× RT Master Mix.

qRT-PCR validation

Real-time PCR was performed with Color SYBR Green qPCR Master Mix ROX2 plus (EZBiosicence, USA) on Roche LightCycler®480. Each reaction was set for 10 μL: 5 μL 2× Color Green qPCR Master Mix, 0.2 μL Forward Primer, 0.2 μL Reverse Primer, 3.6 μL RNase Free dH$_2$O and 1 μL Product from RT reaction. All experiments were repeated three times, and relative gene expression was calculated using the 2-ΔΔCt method. The sequences of gene-specific primers are listed in Table 1.
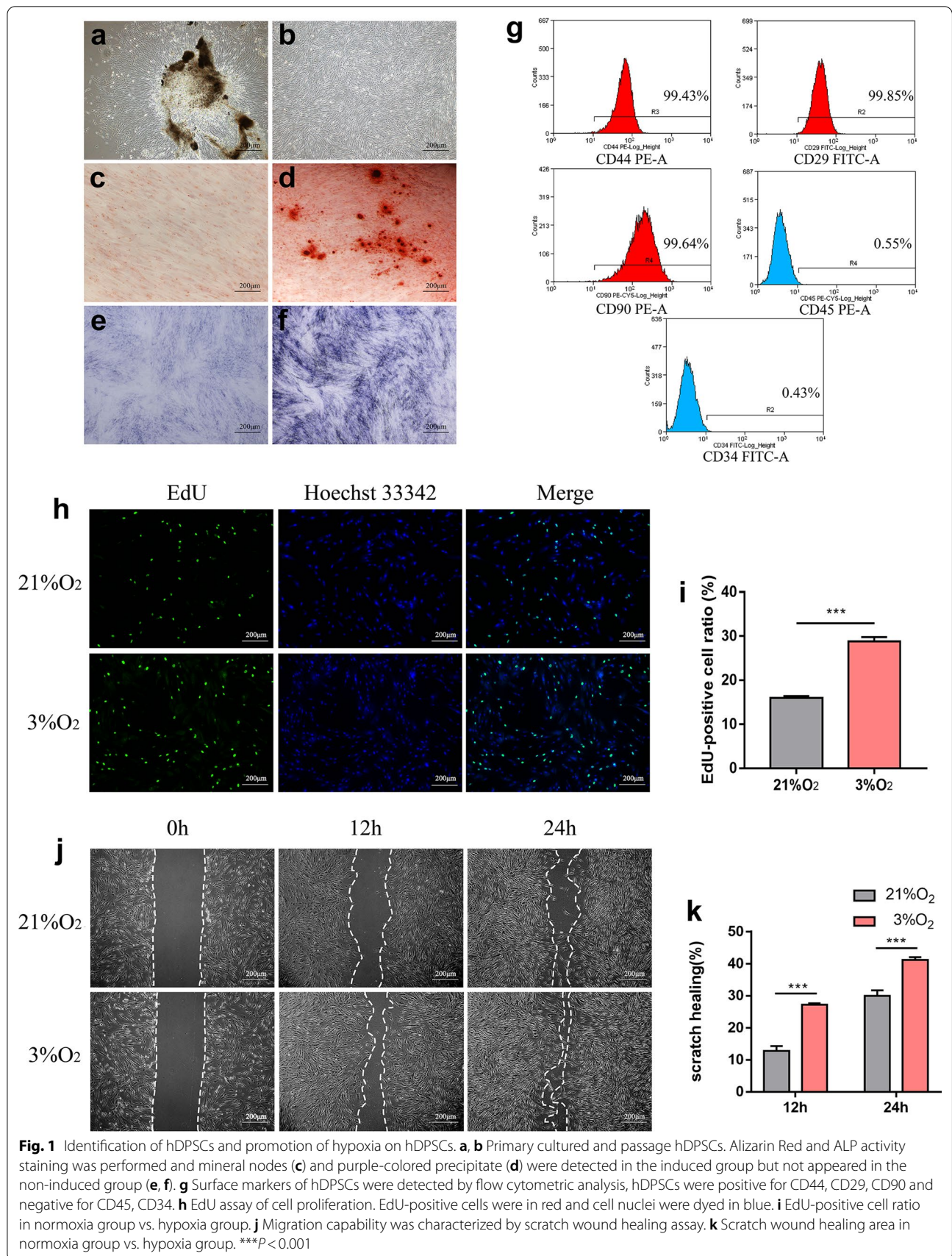
Western blot analysis

Cells of each group were lysed using the RIPA lysis buffer with protease inhibitors and the total protein contents were quantitated by the BCA assay (Beyotime, Shanghai, China). The samples were separated by 8% SDS-PAGE and then were transferred onto polyvinylidene difluoride (PVDF) membranes. The PVDF membranes were blocked in protein-free rapid blocking buffer (Epizyme Biomedical Technology, Shanghai, China) for 15 min and then incubated overnight at 4 °C with primary antibodies against β-actin, AKT, p-AKT, PI3K, p-PI3K (Bioss, Beijing, China), which diluted at 1: 1000. Then, the PVDF membranes were incubated with corresponding secondary antibodies (Proteintech, China). The bands were detected using ECL reagents (Biosharp, China). The densities of protein bands were quantified by Image J software.

Results

hDPSCs exhibit higher proliferation capacity under hypoxia

Primary hDPSCs were successfully isolated from dental pulp tissue and passaged (Fig. 1a, b). The results of staining showed that hDPSCs processed differential capacity (Fig. 1c–f). Flow cytometry results showed that hDPSCs were positive for stem cell markers CD44, CD29 and CD90 but negative for hematopoietic cell markers including CD45 and CD34 (Fig. 1g). Then, the migration and proliferation were, respectively, detected by scratch wound healing assay and EdU assay. As indicated by Fig. 1h–k, hDPSCs proliferated and migrated faster under hypoxia compared with normoxia.

Zeng *et al. Stem Cell Research & Therapy* (2022) 13:286

Page 5 of 16

Fig. 1 Identification of hDPSCs and promotion of hypoxia on hDPSCs. **a**, **b** Primary cultured and passage hDPSCs. Alizarin Red and ALP activity staining was performed and mineral nodes (**c**) and purple-colored precipitate (**d**) were detected in the induced group but not appeared in the non-induced group (**e**, **f**). **g** Surface markers of hDPSCs were detected by flow cytometric analysis, hDPSCs were positive for CD44, CD29, CD90 and negative for CD45, CD34. **h** EdU assay of cell proliferation. EdU-positive cells were in red and cell nuclei were dyed in blue. **i** EdU-positive cell ratio in normoxia group vs. hypoxia group. **j** Migration capability was characterized by scratch wound healing assay. **k** Scratch wound healing area in normoxia group vs. hypoxia group. ***$P < 0.001$

Zeng *et al. Stem Cell Research & Therapy*   (2022) 13:286

Page 6 of 16

Fig. 2 Identification and characterization of novel lncRNAs in hDPSCs under hypoxia. **a** Pipeline for novel lncRNAs identification. **b** Venn diagram showed the intersection of protein-coding potential analysis by CNCI, CPC, CPAT and Pfam-scan. **c** Transcript length distribution of known lncRNA, novel lncRNA and protein coding gene. **d** Distribution of exon number in known lncRNA, novel lncRNA and protein coding gene. **e** Expression level of known lncRNA, novel lncRNA and protein coding gene

Zeng *et al. Stem Cell Research & Therapy*   (2022) 13:286

Page 7 of 16

Identification and characterization of novel lncRNA in hDPSCs under hypoxia

To identify the lncRNAs involved in the hypoxic process, the RNA-Seq data was reanalyzed. According to pipeline (Fig. 2a), novel lncRNAs that may play an important role in regulating hDPSCs under hypoxia were obtained. Compared to the references in GENCODE database, 16,498 protein coding genes, 1351 known lncRNAs were obtained. According to the exon number and transcript length, we further filtered and acquired 1600 potential novel lncRNA transcripts. Then, the coding potential of transcripts was analyzed by CPC, CNCI, CPAT and Pfam-scan, and finally 496 novel lncRNAs that met the definition of lncRNA were obtained (Fig. 2b). Briefly, the novel lncRNAs show longer transcript length, fewer exons, lower coding potential and lower FPKM values, consistent with the characterization of lncRNA as previously reported (Fig. 2c–e)[32].

Differential expression analysis and validation of novel lncRNAs

A total of 1684 differentially expressed genes (DEGs), 135 differentially expressed known lncRNAs and 45 differentially expressed novel lncRNAs (DENLs) were screened under hypoxia compared with normoxia, shown in the volcano plot and heatmap (Fig. 3a–c). Details of the differential expression analyses are provided in Additional file 2. The differential expression of novel lncRNAs was validated by RT-qPCR. The expression levels of novel lncRNAs calculated from qRT-PCR results were similar to those from RNA-Seq data (Fig. 3d). Among them, MSTRG.20649.1 was stably upregulated under hypoxia compared with normoxia. Therefore, we treated MSTRG.20649.1 as the key novel lncRNA that may regulate proliferation and migration of hDPSCs under hypoxia, designated it as lncRNA HRL-SC (hypoxia-responsive lncRNA in stem cells).

Identification, location and expression pattern analysis of lncRNA HRL-SC

lncRNA HRL-SC is an intronic lncRNA and located in chromosome 3, from locus 48,566,603 to locus 48,567,458 (Additional file 1: Fig. 1a). RNA-FISH test was performed to examine the subcellular location of HRL-SC, which showed that HRL-SC was mainly located in the cytoplasm of hDPSCs under hypoxia but in the nucleus under normoxia (Fig. 4a). To further understand the expression pattern of HRL-SC in hDPSCs, we detected its expression levels under different oxygen concentrations with different exposure durations. The RT-qPCR results showed that HRL-SC had a higher expression level in $3\%O_2$ when

compared with $1\%O_2$, $5\%O_2$ and $21\%O_2$ (Fig. 4b). In addition, the dynamic expression levels of HRL-SC were also observed in hDPSCs under hypoxia (Fig. 4c).

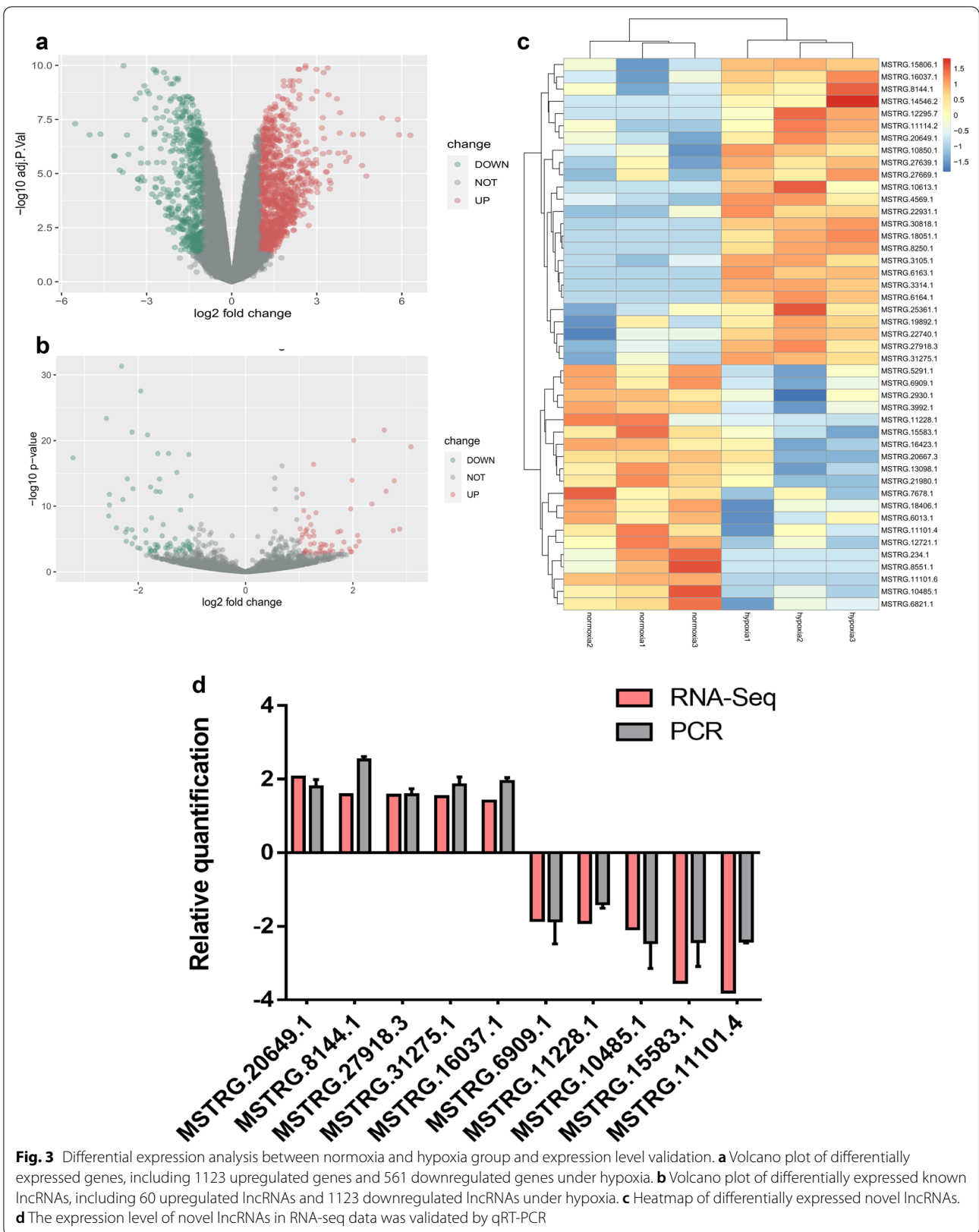### Target genes and pathway analysis of lncRNA HRL-SC

To understand the function of key novel lncRNA, the target prediction of HRL-SC was performed, and the results showed that there was a total of 592 potential target genes identified from DEGs. The functions of target genes were analyzed through functional enrichment analysis. As shown in Fig. 5a, target genes of HRL-SC were significantly enriched in the response to hypoxia and the oxidation–reduction process. GSEA results confirmed this association that the differential expression of HRL-SC was associated with hypoxia conditions (Fig. 5b). In addition, pathway analysis indicated that PI3K/AKT signaling pathway, metabolic pathways and the other related pathway were identified (Fig. 5c). The expression analysis of target genes in pathways are shown in Fig. 5d. Among these, PDK1 was the most significantly differentially expressed target gene of HRL-SC in the PI3K/AKT signaling pathway (Fig. 5d). The details for prediction of target genes and functional annotation analysis are provided in Additional files 3, 4, 5.
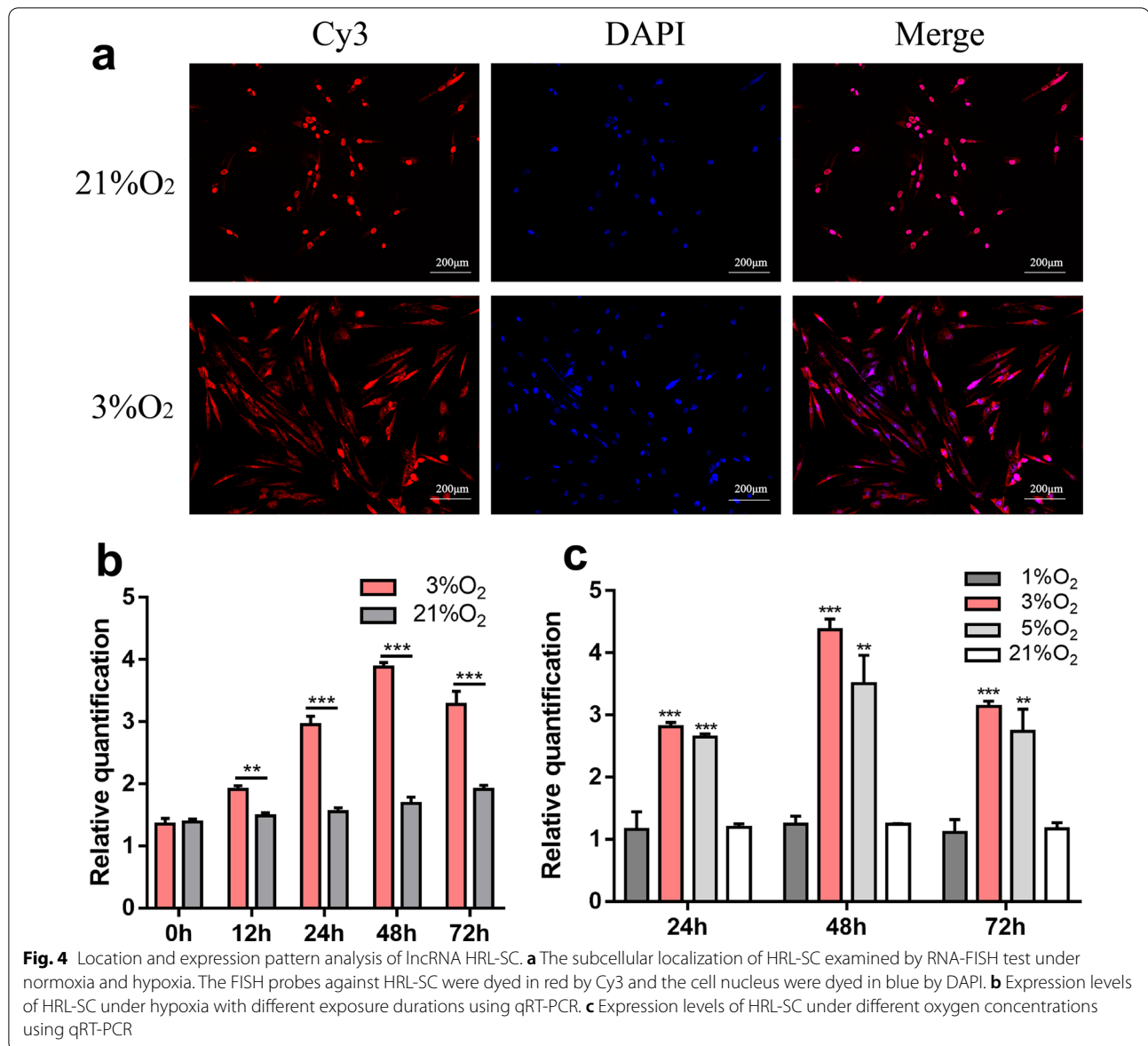
Knockdown of lncRNA HRL-SC inhibited the proliferation and migration of hDPSCs

To confirm the role of HRL-SC in regulating proliferation and migration of hDPSCs, siRNA-mediated knockdown was performed and the expression levels of HRL-SC significantly decreased (Additional file 1: Fig. 1b). Moreover, the EdU assay showed that the cell proliferation rate in siRNA group was significantly lower than blank control and siRNA-ctrl group (Fig. 6a). Knockdown of HRL-SC inhibited proliferation of hDPSCs under hypoxia. Furthermore, the knockdown of HRL-SC also inhibited cell migration of hDPSCs. As shown in Fig. 6a, b lower migration rate was observed in siRNA group compared with blank control and siRNA-ctrl group.

Knockdown of lncRNA HRL-SC inhibited the proliferation and migration of hDPSCs through the PI3K/AKT signaling pathway

Our previous study demonstrated that PI3K/AKT signaling pathway-related marker proteins were increased in hDPSCs under hypoxia. Besides, the expression levels of PDK1, one of the potential target genes of HRL-SC, and HIF-1α increased during hypoxia exposure but decreased after the knockdown of HRL-SC (Fig. 7a–c). Combining with the results from pathway analysis of HRL-SC target genes, we speculated that HRL-SC may regulate proliferation and migration of hDPSCs
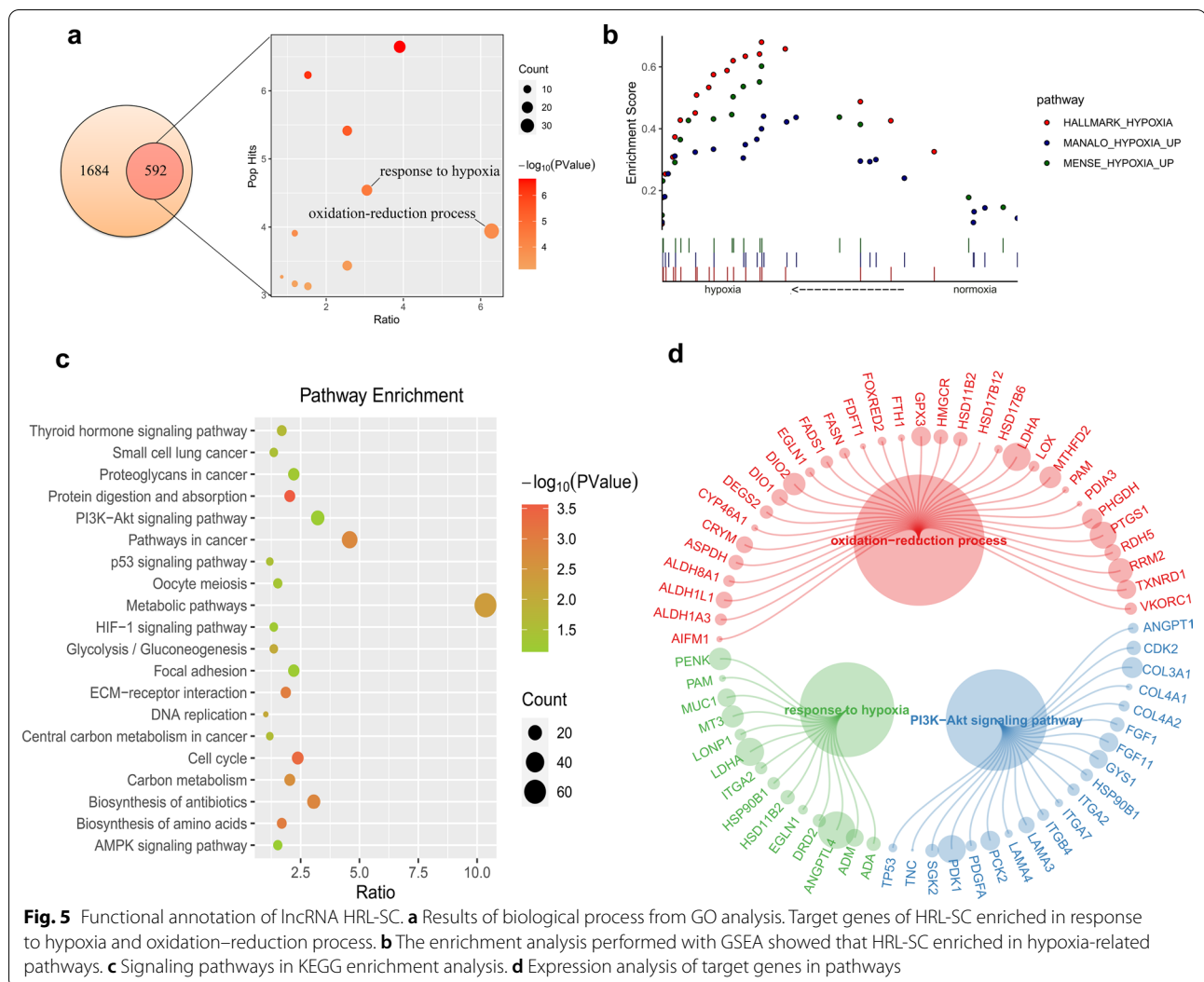
Zeng *et al. Stem Cell Research & Therapy*   (2022) 13:286

Page 8 of 16

Fig. 3 Differential expression analysis between normoxia and hypoxia group and expression level validation. **a** Volcano plot of differentially expressed genes, including 1123 upregulated genes and 561 downregulated genes under hypoxia. **b** Volcano plot of differentially expressed known lncRNAs, including 60 upregulated lncRNAs and 1123 downregulated lncRNAs under hypoxia. **c** Heatmap of differentially expressed novel lncRNAs. **d** The expression level of novel lncRNAs in RNA-seq data was validated by qRT-PCR

Zeng *et al. Stem Cell Research & Therapy*   (2022) 13:286

Page 9 of 16

Fig. 4 Location and expression pattern analysis of lncRNA HRL-SC. **a** The subcellular localization of HRL-SC examined by RNA-FISH test under normoxia and hypoxia. The FISH probes against HRL-SC were dyed in red by Cy3 and the cell nucleus were dyed in blue by DAPI. **b** Expression levels of HRL-SC under hypoxia with different exposure durations using qRT-PCR. **c** Expression levels of HRL-SC under different oxygen concentrations using qRT-PCR

through PI3K/AKT signaling pathway. To test this hypothesis, the expression levels of PI3K/AKT-related marker proteins were examined after the knockdown of HRL-SC. The results revealed that knockdown of HRL-SC decreased ratios of p-AKT/AKT and p-PI3K/PI3K, inhibiting the activation of the signaling pathway (Fig. 7d–f). Next, we added AKT activator SC79 into the medium when cell transfection was performed. The western bolt analysis showed that SC79 enhanced AKT phosphorylation and upregulated the expression of p-AKT (Fig. 8a, b). Furthermore, the EdU assay and scratch wound healing assay revealed that SC79 promoted the proliferation and migration of hDPSCs under hypoxia after knockdown of HRL-SC (Fig. 8c–f). In general, the results demonstrated that HRL-SC regulated the proliferation and migration of hDPSCs under hypoxia through the PI3K/AKT signaling pathway.

Discussion

hDPSCs are typically isolated and cultured under normoxia, which differs from the stem cell microenvironment [33]. As hDPSCs proliferate during extended culture under normoxia, senescence-associated β-galactosidase levels increase, which is a premature senescence phenotype of stem cells [34, 35]. Accumulated injury caused by high oxygen tension can be damaging to stem cells [36]. Although there are some
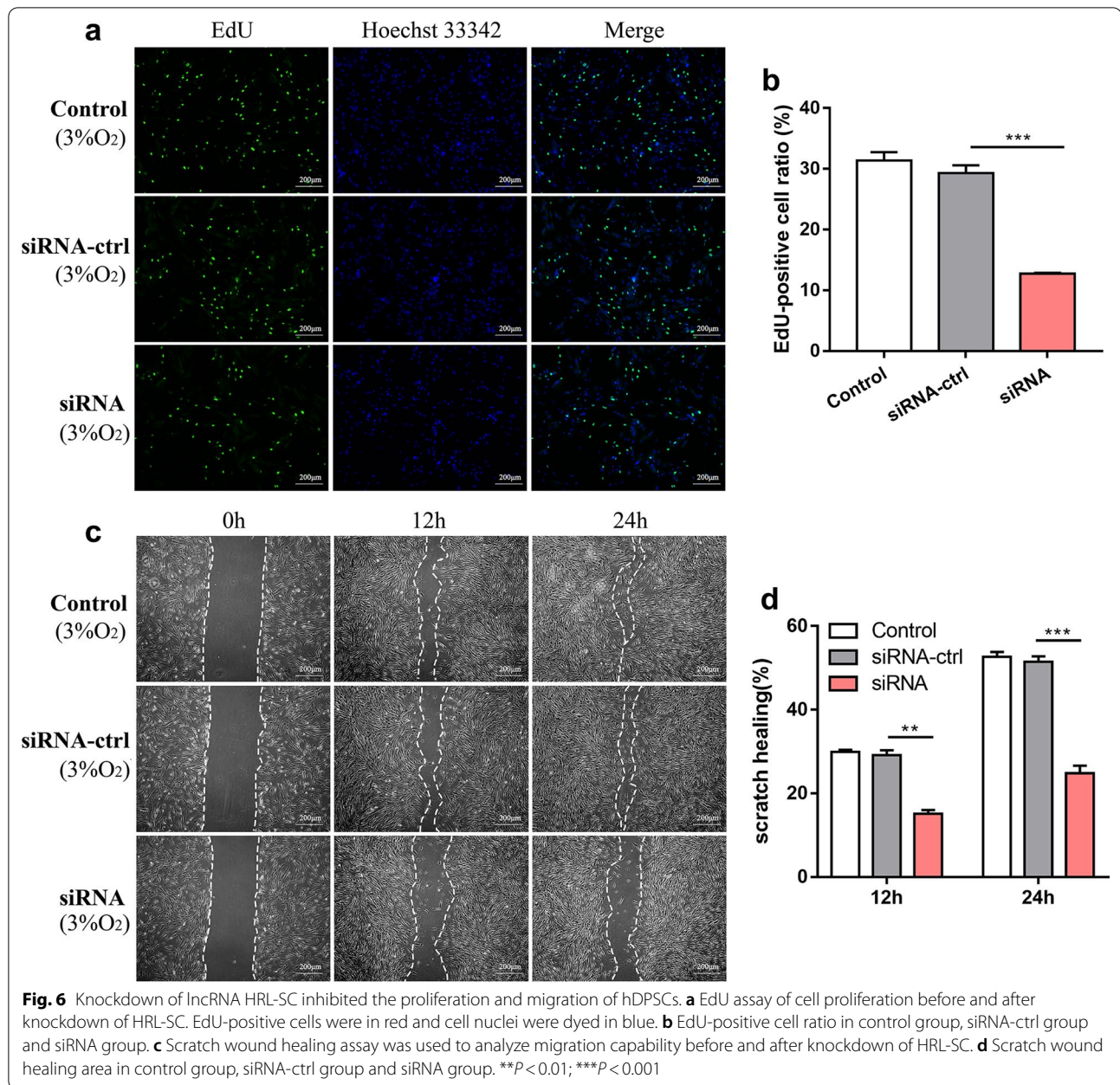
Zeng *et al. Stem Cell Research & Therapy* (2022) 13:286

Page 10 of 16

Fig. 5 Functional annotation of lncRNA HRL-SC. **a** Results of biological process from GO analysis. Target genes of HRL-SC enriched in response to hypoxia and oxidation–reduction process. **b** The enrichment analysis performed with GSEA showed that HRL-SC enriched in hypoxia-related pathways. **c** Signaling pathways in KEGG enrichment analysis. **d** Expression analysis of target genes in pathways

controversies related to the effect of hypoxia on hDP-SCs, most studies have suggested that low oxygen tension promotes the proliferation and migration of hDPSCs [15, 16, 30]. The oxygen concentration has been set from 1 to 5% in the studies of hDPSCs in vitro under hypoxia, and evidence points to 3% O_2 as the ideal concentration [15, 17, 30, 37], probably because the oxygen concentration of rat incisor pulp is approximately 3% [12]. Similar results were also observed in our study. When cultured under hypoxia, scratch wound healing and EdU assays revealed that hDPSCs exhibited higher levels of proliferation and migration than those cultured under normoxia.

Studies on HRL have mostly focused on tumor stem cells [38–40]. For example, the novel lncRNA KB-1980E6.3 was identified in breast cancer stem cells under hypoxia and was suggested to maintain stemness through the lncRNA KB-1980E6.3/IGF2BP1/c-Myc axis [40]. The
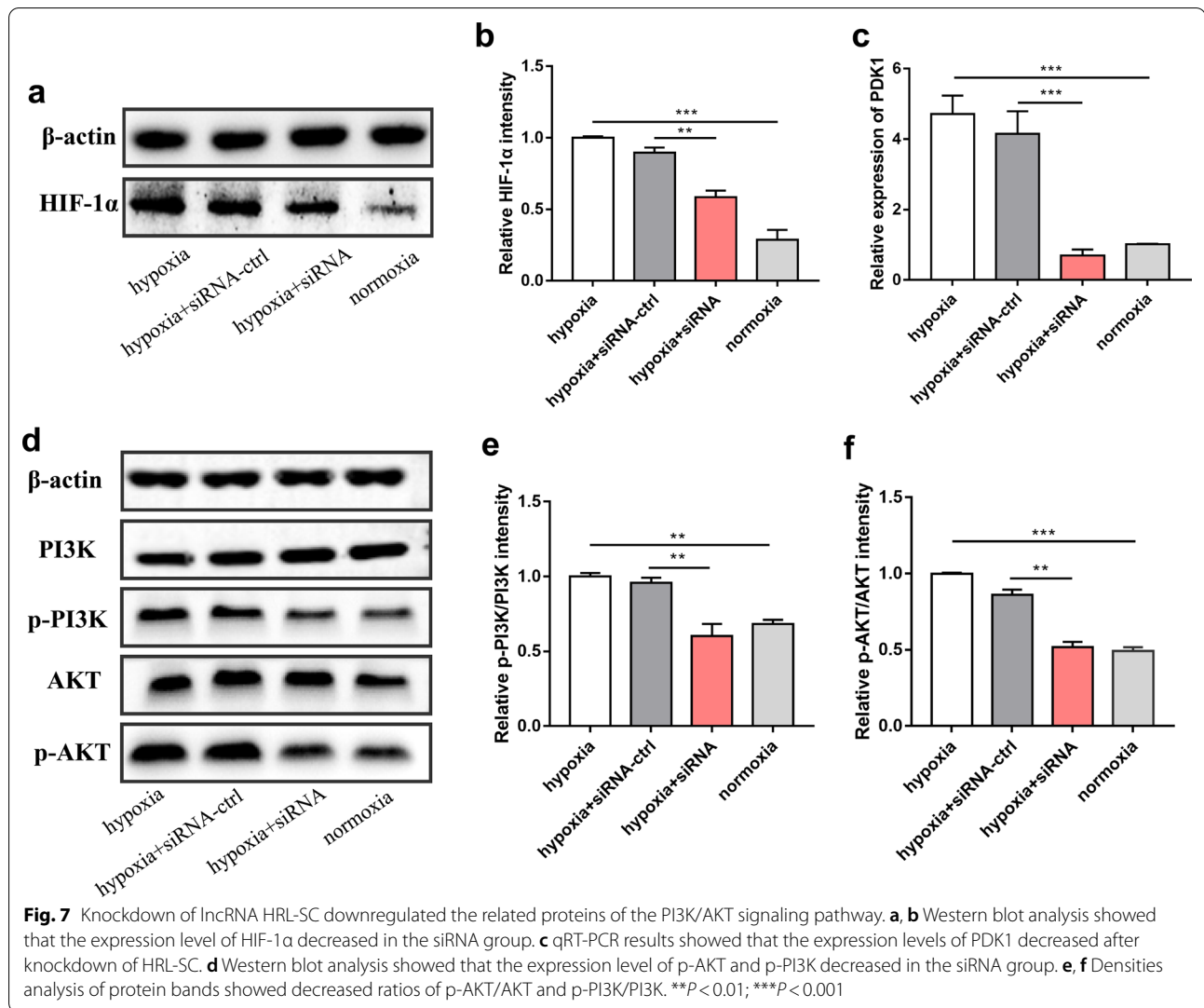
underlying mechanism of HRL in hDPSCs, which is also promoted by hypoxia, remains unclear.

In the present study, we identified 496 unannotated novel lncRNAs from RNA-Seq data. By analyzing the exon number, chromosome distribution, FPKM values and coding ability, we found that the novel lncRNAs we identified conformed to the basic characteristics of lncRNAs. Based on the results of differential expression analysis and qRT-PCR, we screened the key novel lncRNAs that may play an important role in promoting the proliferation and migration of hDPSCs under hypoxia. Functional enrichment analysis revealed that HRL-SC was involved in the response to hypoxia and other hypoxia-related biological processes. The biological mechanisms of lncRNAs are associated with their subcellular localization [22]. Some lncRNAs distributed in the nucleus are generally involved in transcription or epigenetic regulation [41]. The other lncRNAs

Zeng *et al. Stem Cell Research & Therapy*   (2022) 13:286

Page 11 of 16

Fig. 6 Knockdown of lncRNA HRL-SC inhibited the proliferation and migration of hDPSCs. **a** EdU assay of cell proliferation before and after knockdown of HRL-SC. EdU-positive cells were in red and cell nuclei were dyed in blue. **b** EdU-positive cell ratio in control group, siRNA-ctrl group and siRNA group. **c** Scratch wound healing assay was used to analyze migration capability before and after knockdown of HRL-SC. **d** Scratch wound healing area in control group, siRNA-ctrl group and siRNA group. $**P < 0.01$; $***P < 0.001$

distributed in the cytoplasm are related to posttranscriptional regulation, including splicing, editing, transport, translation, degradation of mRNA and miRNA sponges [42]. The RNA FISH revealed that HRL-SC was located in the cell nucleus under normoxia but accumulated in the cell cytoplasm under hypoxia. The increased expression level and change in location of HRL-SC were observed from normoxia to hypoxia. We then determined the expression level of HRL-SC at different oxygen concentrations with different treatment times to observe the expression pattern of HRL-SC. Interestingly, dynamic expression levels of HRL-SC were observed in hDPSCs under hypoxia. Expression of HRL-SC was highest at 3% O_2, which is considered the ideal oxygen concentration for in vitro culture of hDP-SCs. However, HRL-SC expression at 1% O_2 was lower and similar to that in normoxia, probably because 1% O_2 was not the ideal oxygen concentration for hDPSCs, and hypoxic injury that affects cell metabolism might occur under severe hypoxia [43]. Combined with the

Fig. 7 Knockdown of lncRNA HRL-SC downregulated the related proteins of the PI3K/AKT signaling pathway. **a**, **b** Western blot analysis showed that the expression level of HIF-1α decreased in the siRNA group. **c** qRT-PCR results showed that the expression levels of PDK1 decreased after knockdown of HRL-SC. **d** Western blot analysis showed that the expression level of p-AKT and p-PI3K decreased in the siRNA group. **e**, **f** Densities analysis of protein bands showed decreased ratios of p-AKT/AKT and p-PI3K/PI3K. **$P < 0.01$; ***$P < 0.001$

bioinformatic analysis, we demonstrated that HRL-SC functions as a hypoxia-responsive lncRNA to regulate hDPSCs.

To inhibit the functions of HRL-SC in hDPSCs under hypoxia, we used siRNA, an efficient silencing method for cytoplasmic lncRNA [44], to knockdown its expression. Knockdown of HRL-SC inhibited both the proliferation and migration of hDPSCs under hypoxia, but the negative control siRNA did not produce harmful effects. Physiological oxygen tension under hypoxic conditions is a necessary condition for the activation of hypoxia-inducible factor-1α (HIF-1α) to maintain and protect stem cells [53, 54]. The energy supply of stem cells under hypoxia switches from oxidative phosphorylation to the glycolytic pathway, which is induced by HIF-1α [45]. It has also been reported that activation of AKT enhances HIF-1α translation. AKT is the core regulator of PI3K/AKT signaling, which is activated upon phosphorylation

[46]. AKT phosphorylation is initiated by PI3K, which recruits AKT and PDK1 to the cell membrane, and PDK1 then phosphorylates AKT site Thr308 to partially activate AKT [47]. PI3K/AKT is constitutively activated in stem cells under hypoxia [48], and alteration of PI3K/AKT phosphorylation was also observed in our results. Some lncRNAs were demonstrated to regulate the activity of stem cells via the PI3K/AKT signaling pathway. For example, it was observed that lncRNA AK015322 promotes the proliferation of hair follicle stem cells via the PI3K/AKT signaling pathway [49]. The decreased expression of p-AKT and p-PI3K showed that HRL-SC might initiate constitutive activation of PI3K/AKT signaling in a variety of ways. Similar to our study, knockdown of lnc-ORA, a novel lncRNA that was identified from RNA-Seq data, reduced the expression of p-AKT and p-PI3K in adipose tissue, and lnc-ORA was considered to regulate adipocyte differentiation via the PI3K/AKT signaling
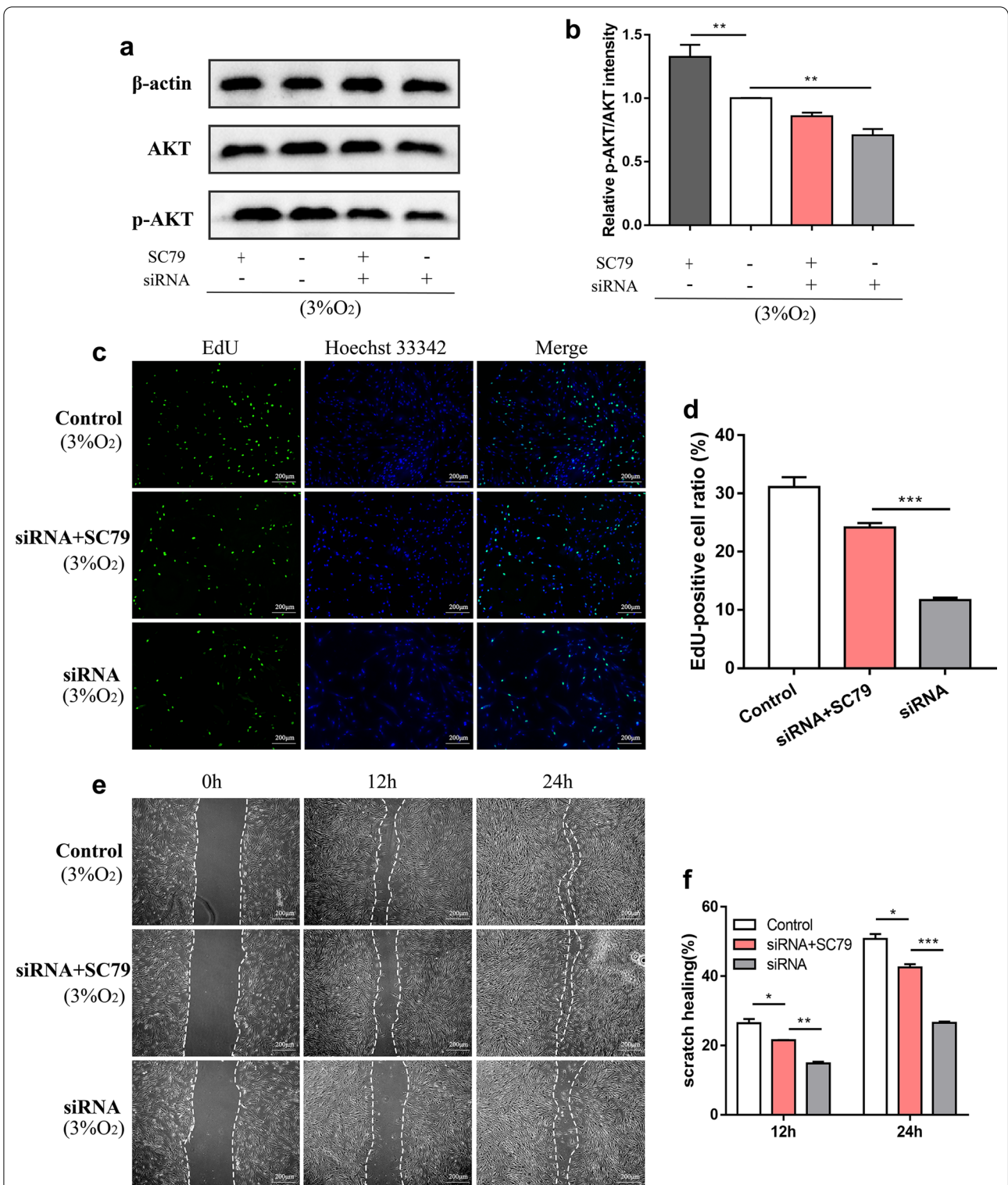
Zeng *et al. Stem Cell Research & Therapy*   (2022) 13:286

Page 13 of 16

Fig. 8 SC79 partially rescued the inhibitory effect of proliferation and migration caused by the knockdown of HRL-SC. **a**, **b** Western blot analysis showed that SC79 increased expression of p-AKT. **c** EdU assay of cell proliferation before and after addition of SC79. EdU-positive cells were in red and cell nuclei were dyed in blue. **d** EdU-positive cell ratio in control group, siRNA + SC79 group and siRNA group. **e** Scratch wound healing assay was performed to analyze migration capability before and after the addition of SC79. **f** Scratch wound healing area in control group, siRNA + SC79 group and siRNA group. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Zeng *et al. Stem Cell Research & Therapy*   (2022) 13:286

Page 14 of 16

pathway [50]. Because PDK1 was one of the potential target genes of HRL-SC and its expression decreased after knockdown of HRL-SC, one possibility is that HRL-SC modulated PDK1 to activate the PI3K/AKT signaling pathway [51].

For further validation, we added SC79 to the medium after knockdown of HRL-SC. SC79 is an AKT activator that induces the phosphorylation of AKT to activate the PI3K/AKT signaling pathway [52]. The activation of p-AKT by SC79 was confirmed. As the results showed, SC79 partially rescued the inhibitory effect of HRL-SC knockdown on proliferation and migration in hDPSCs under hypoxia. Therefore, we considered that the novel HRL-SC promotes the proliferation and migration of hDPSCs under hypoxia via the PI3K/AKT signaling pathway.

There are still several potential limitations. For example, the binding sites and target genes of HRL-SC still need plenty of experiments to confirm. In addition, there is no perfect cell model with the ability to completely mimic all characteristics of the hDPSCs in human dental pulp, so the experiment results may not be entirely consistent with the actual situation. The specific regulatory mechanism will be investigated in our future studies.

Conclusions

In conclusion, we identified a novel HRL-SC from RNA-Seq data of hDPSCs under hypoxia compared with normoxia. HRL-SC is a hypoxia-responsive lncRNA that promotes the proliferation and migration of hDPSCs through the PI3K/AKT signaling pathway. These findings contribute to an understanding of the mechanism of lncRNA in hDPSCs under hypoxia and to the application of hDPSCs in pulp regeneration.

Abbreviations

HRL: Hypoxia-responsive lncRNA; CNCI: Coding-Non-Coding-Index; CPAT: Coding Potential Assessment Tool; CPC: Coding Potential Calculator; DAVID: Database for Annotation Visualization and Integrated Discovery; DEGs: Differentially expressed genes; DENLs: Differentially expressed novel lncRNAs; DMEM: Dulbecco modified eagle's medium; FBS: Fetal bovine serum; FISH: Fluorescence in situ hybridization; GO: Gene ontology; GSEA: Gene set enrichment analysis; hDPSCs: Human dental pulp stem cells; HIF: Hypoxia inducible factor; KEGG: Kyoto Encyclopedia of Genes and Genomes; lncRNAs: Long noncoding RNAs; Oct-4: Octamer-binding protein 4; PVDF: Polyvinylidene difluoride; siRNA: Small interfering RNA; SOX2: SRY-Box transcription factor 2.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13287-022-02970-5.

Additional file 1: Fig. S1. The sequence information of lncRNA HRL-SC and validation of siRNA-mediated knockdown using qRT-PCR.

Additional file 2. Details of differentially expressed mRNAs, known lncRNAs and novel lncRNAs under hypoxia compared with normoxia.

Additional file 3. Details of target genes of HRL-SC.

Additional file 4. Details of GO analysis for HRL-SC.

Additional file 5. Details of KEGG pathway analysis for HRL-SC.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Nanfang Hospital, Southern Medical University. Written informed consent was obtained from all adult patients. For the minor population, a statement on informed consent was obtained from their parents.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, et al. Stem cell properties of human dental pulp stem cells. J Dent Res. 2002;81:531–5. https://doi.org/10.1177/154405910208100806.
2. Shi X, Mao J, Liu Y. Pulp stem cells derived from human permanent and deciduous teeth: biological characteristics and therapeutic applications. Stem Cells Transl Med. 2020;9:445–64.
3. Huang GT-J, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res. 2009;88:792–806. https://doi.org/10.1177/0022034509340867.
4. Nuti N, Corallo C, Chan BMF, Ferrari M, Gerami-Naini B. Multipotent differentiation of human dental pulp stem cells: a literature review. Stem Cell Rev Rep. 2016;12:511–23. https://doi.org/10.1007/s12015-016-9661-9.
5. Xie Z, Shen Z, Zhan P, Yang J, Huang Q, Huang S, et al. Functional dental pulp regeneration: basic research and clinical translation. Int J Mol Sci. 2021;22:8991.
6. Yamada Y, Nakamura-Yamada S, Kusano K, Baba S. Clinical potential and current progress of dental pulp stem cells for various systemic diseases in regenerative medicine: a concise review. Int J Mol Sci. 2019;20:1132.

Zeng *et al. Stem Cell Research & Therapy*   (2022) 13:286

Page 15 of 16

7. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells. 1978;4:7–25.

8. Mas-Bargues C, Sanz-Ros J, Román-Domínguez A, Inglés M, Gimeno-Mallench L, El Alami M, et al. Relevance of oxygen concentration in stem cell culture for regenerative medicine. Int J Mol Sci. 2019;20:E1195.

9. Csete M. Oxygen in the cultivation of stem cells. Ann N Y Acad Sci. 2005;1049:1–8. https://doi.org/10.1196/annals.1334.001.

10. Mohyeldin A, Garzón-Muvdi T, Quiñones-Hinojosa A. Oxygen in stem cell biology: a critical component of the stem cell niche. Cell Stem Cell. 2010;7:150–61.

11. Peck SH, Bendigo JR, Tobias JW, Dodge GR, Malhotra NR, Mauck RL, et al. Hypoxic preconditioning enhances bone marrow-derived mesenchymal stem cell survival in a low oxygen and nutrient-limited 3D microenvironment. Cartilage. 2021;12:512–25. https://doi.org/10.1177/1947603519841675.

12. Yu CY, Boyd NM, Cringle SJ, Alder VA, Yu DY. Oxygen distribution and consumption in rat lower incisor pulp. Arch Oral Biol. 2002;47:529–36.

13. Ciccone MM, Cortese F, Gesualdo M, Carbonara S, Zito A, Ricci G, et al. Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. Mediat Inflamm. 2013;2013:1–11.

14. Eliasson P, Jönsson J-I. The hematopoietic stem cell niche: low in oxygen but a nice place to be. J Cell Physiol. 2010;222:17–22. https://doi.org/10.1002/jcp.21908.

15. Iida K, Takeda-Kawaguchi T, Tezuka Y, Kunisada T, Shibata T, Tezuka K. Hypoxia enhances colony formation and proliferation but inhibits differentiation of human dental pulp cells. Arch Oral Biol. 2010;55:648–54.

16. Aranha AMF, Zhang Z, Neiva KG, Costa CAS, Hebling J, Nör JE. Hypoxia enhances the angiogenic potential of human dental pulp cells. J Endod. 2010;36:1633–7.

17. Sakdee JB, White RR, Pagonis TC, Hauschka PV. Hypoxia-amplified proliferation of human dental pulp cells. J Endod. 2009;35:818–23.

18. Zhou Y, Fan W, Xiao Y. The effect of hypoxia on the stemness and differentiation capacity of PDLC and DPC. BioMed Res Int. 2014;2014:1–7.

19. Vukovic M, Sepulveda C, Subramani C, Guitart AV, Mohr J, Allen L, et al. Adult hematopoietic stem cells lacking Hif-1α self-renew normally. Blood. 2016;127:2841–6.

20. Mathieu J, Zhou W, Xing Y, Sperber H, Ferreccio A, Agoston Z, et al. Hypoxia-inducible factors have distinct and stage-specific roles during reprogramming of human cells to pluripotency. Cell Stem Cell. 2014;14:592–605.

21. Quinodoz S, Guttman M. Long noncoding RNAs: an emerging link between gene regulation and nuclear organization. Trends Cell Biol. 2014;24:651–63.

22. Bridges MC, Daulagala AC, Kourtidis A. LNCcation: lncRNA localization and function. J Cell Biol. 2021;220:e202009045.

23. Aich M, Chakraborty D. Role of lncRNAs in stem cell maintenance and differentiation. Curr Top Dev Biol. 2020;138:73–112.

24. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet. 2009;10:155–9.

25. Xu S, Tang L, Liu Z, Luo C, Cheng Q. Hypoxia-related lncRNA correlates with prognosis and immune microenvironment in lower-grade glioma. Front Immunol. 2021;12:731048. https://doi.org/10.3389/fimmu.2021.731048/full.

26. Shi H, Wang M, Sun J, Wang H, Li Y, Chen B, et al. A novel long noncoding RNA FAF inhibits apoptosis via upregulating FGF9 through PI3K/AKT signaling pathway in ischemia–hypoxia cardiomyocytes. J Cell Physiol. 2019;234:21973–87. https://doi.org/10.1002/jcp.28760.

27. He J, Huang Y, Liu J, Ge L, Tang X, Lu M, et al. Hypoxic conditioned promotes the proliferation of human olfactory mucosa mesenchymal stem cells and relevant lncRNA and mRNA analysis. Life Sci. 2021;265:118861.

28. Meng S-S, Xu X-P, Chang W, Lu Z-H, Huang L-L, Xu J-Y, et al. LincRNA-p21 promotes mesenchymal stem cell migration capacity and survival through hypoxic preconditioning. Stem Cell Res Ther. 2018;9:280. https://doi.org/10.1186/s13287-018-1031-x.

29. Hou J, Wang L, Wu Q, Zheng G, Long H, Wu H, et al. Long noncoding RNA H19 upregulates vascular endothelial growth factor A to enhance mesenchymal stem cells survival and angiogenic capacity by inhibiting miR-199a-5p. Stem Cell Res Ther. 2018;9:109. https://doi.org/10.1186/s13287-018-0861-x.

30. Shi R, Yang H, Lin X, Cao Y, Zhang C, Fan Z, et al. Analysis of the characteristics and expression profiles of coding and noncoding RNAs of human dental pulp stem cells in hypoxic conditions. Stem Cell Res Ther. 2019;10:89. https://doi.org/10.1186/s13287-019-1192-2.

31. Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA, Sonnhammer ELL, et al. Pfam: the protein families database in 2021. Nucleic Acids Res. 2021;49:D412–9.

32. Chen L, Shi G, Chen G, Li J, Li M, Zou C, et al. Transcriptome analysis suggests the roles of long intergenic non-coding RNAs in the growth performance of weaned piglets. Front Genet. 2019;10:196. https://doi.org/10.3389/fgene.2019.00196/full.

33. Labedz-Maslowska A, Bryniarska N, Kubiak A, Kaczmarzyk T, Sekula-Stryjewska M, Noga S, et al. Multilineage differentiation potential of human dental pulp stem cells—impact of 3D and hypoxic environment on osteogenesis in vitro. Int J Mol Sci. 2020;21:6172.

34. Alaidaroos NYA, Alraies A, Waddington RJ, Sloan AJ, Moseley R. Differential SOD2 and GSTZ1 profiles contribute to contrasting dental pulp stem cell susceptibilities to oxidative damage and premature senescence. Stem Cell Res Ther. 2021;12:142. https://doi.org/10.1186/s13287-021-02209-9.

35. Asghari M, Nasoohi N, Hodjat M. High glucose promotes the aging of human dental pulp cells through Wnt/beta-catenin signaling. Dent Med Probl. 2021;58:39–46.

36. Mas-Bargues C, Sanz-Ros J, Román-Domínguez A, Gimeno-Mallench L, Inglés M, Viña J, et al. Extracellular vesicles from healthy cells improves cell function and stemness in premature senescent stem cells by miR-302b and HIF-1α activation. Biomolecules. 2020;10:957.

37. Werle SB, Chagastelles P, Pranke P, Casagrande L. Hypoxia upregulates the expression of the pluripotency markers in the stem cells from human deciduous teeth. Clin Oral Investig. 2019;23:199–207. https://doi.org/10.1007/s00784-018-2427-9.

38. Li J, Liao T, Liu H, Yuan H, Ouyang T, Wang J, et al. Hypoxic glioma stem cell-derived exosomes containing Linc01060 promote progression of glioma by regulating the MZF1/c-Myc/HIF-1α. Cancer Res. 2020;81:114–28. https://doi.org/10.1158/0008-5472.CAN-20-2270.

39. García-Venzor A, Mandujano-Tinoco EA, Ruiz-Silvestre A, Sánchez JM, Lizarraga F, Zampedri C, et al. lncMat2B regulated by severe hypoxia induces cisplatin resistance by increasing DNA damage repair and tumor-initiating population in breast cancer cells. Carcinogenesis. 2020;41:1485–97.

40. Zhu P, He F, Hou Y, Tu G, Li Q, Jin T, et al. A novel hypoxic long noncoding RNA KB-1980E6.3 maintains breast cancer stem cell stemness via interacting with IGF2BP1 to facilitate c-Myc mRNA stability. Oncogene. 2021;40:1609–27.

41. Yao R-W, Wang Y, Chen L-L. Cellular functions of long noncoding RNAs. Nat Cell Biol. 2019;21:542–51.

42. Rashid F, Shah A, Shan G. Long non-coding RNAs in the cytoplasm. Genomics Proteomics Bioinform. 2016;14:73–80.

43. Fu X, Feng Y, Shao B, Zhang Y. Taxifolin protects dental pulp stem cells under hypoxia and inflammation conditions. Cell Transplant. 2021;30:096368972110344. https://doi.org/10.1177/09636897211034452.

44. Soares RJ, Maglieri G, Gutschner T, Diederichs S, Lund AH, Nielsen BS, et al. Evaluation of fluorescence in situ hybridization techniques to study long non-coding RNA expression in cultured cells. Nucleic Acids Res. 2018;46:e4.

45. Kim J, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab. 2006;3:177–85.

46. Hemmings BA, Restuccia DF. PI3K-PKB/Akt pathway. Cold Spring Harb Perspect Biol. 2012;4:a011189–a011189. https://doi.org/10.1101/cshperspect.a011189.

47. Xie Y, Shi X, Sheng K, Han G, Li W, Zhao Q, et al. PI3K/Akt signaling transduction pathway, erythropoiesis and glycolysis in hypoxia (Review). Mol Med Rep. 2018;19:783–91.

48. Liu F, Huang X, Luo Z, He J, Haider F, Song C, et al. Hypoxia-activated PI3K/Akt inhibits oxidative stress via the regulation of reactive oxygen species in human dental pulp cells. Oxid Med Cell Longev. 2019;2019:1–10.

49. Cai B, Zheng Y, Ma S, Xing Q, Wang X, Yang B, et al. Long non-coding RNA regulates hair follicle stem cell proliferation and differentiation through PI3K/AKT signal pathway. Mol Med Rep. 2018. https://doi.org/10.3892/mmr.2018.8546.

50. Cai R, Tang G, Zhang Q, Yong W, Zhang W, Xiao J, et al. A novel lnc-RNA, named lnc-ORA, is identified by RNA-Seq analysis, and its knockdown

Zeng *et al. Stem Cell Research & Therapy*   (2022) 13:286

Page 16 of 16

inhibits adipogenesis by regulating the PI3K/AKT/mTOR signaling pathway. Cells. 2019;8:477.

51. Wang XQ, Lo CM, Chen L, Ngan ES-W, Xu A, Poon RY. CDK1-PDK1-PI3K/Akt signaling pathway regulates embryonic and induced pluripotency. Cell Death Differ. 2017;24:38–48.

52. Luan Q, Pan L, He D, Gong X, Zhou H. SC79, the AKT activator protects cerebral ischemia in a rat model of ischemia/reperfusion injury. Med Sci Monit. 2018;24:5391–7.

53. Bhandi S, Al Kahtani A, Mashyakhy M, Alsofi L, Maganur PC, Vishwanathaiah S, et al. Modulation of the dental pulp stem cell secretory profile by hypoxia induction using cobalt chloride. J Pers Med. 2021;11:247. Available from: https://www.mdpi.com/2075-4426/11/4/247.

54. Zehra M, Zubairi W, Hasan A, Butt H, Ramzan A, Azam M, et al. Oxygen generating polymeric nano fibers that stimulate angiogenesis and show efficient wound healing in a diabetic wound model. Int J Nanomedicine. 2020;15:3511–22. Available from: https://www.dovepress.com/oxygen-generating-polymeric-nano-fibers-that-stimulate-angiogenesis-an-peer-reviewed-article-IJN.

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