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RESEARCH ARTICLE

Slow twitch paraspinal muscle dysregulation in adolescent idiopathic scoliosis exhibiting HIF-2α misexpression

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Abstract

Background: Adolescent idiopathic scoliosis (AIS) refers to a three-dimensional spinal deformity which has a typical onset during adolescence. In most cases, the cause of the deformity cannot be clearly identified. Unbalanced paraspinal muscle activity in AIS patients was reported and hypoxia was implicated to regulate myogenesis. This study aims to investigate the association between myogenesis/muscle toning and HIF-αs activity in the pathogenesis of AIS.

Methods: HIF-αs expression was examined by enzyme-linked immunosorbent assay and western blot in paraspinal myoblasts isolated from 18 subjects who underwent deformity correction surgery. QPCR was conducted to measure the gene expression levels of perinatal muscle fiber markers *MYH3*, *MYH8*; slow twitch muscle fiber markers *MHY7*; fast twitch muscle fiber markers *MYH4*; and myogenic regulatory factors *MYF5* and *MYOG*. Slow and fast twitch muscle fiber composition in concave/convex paraspinal musculature of AIS subjects was evaluated by immunostaining of myosin heavy chain type I (MyHC I) and myosin heavy chain type II (MyHC II).

Results: Reduced HIF-2α induction under hypoxia was found in paraspinal myoblast culture of 33% AIS subjects. We detected a suppression of perinatal and slow twitch muscle fiber associated genes, but not fast twitch muscle fiber-associated genes and myogenic regulatory factors in HIF-2α misexpressed AIS myoblasts. Distinct reduction of slow twitch muscle fiber was evidenced in convex paraspinal musculature, suggesting an asymmetric expression of slow twitch muscle fiber in HIF-2α misexpressed AIS patients.

Conclusions: This study indicates an association of abnormal HIF-2α expression in paraspinal myoblasts and a disproportionate slow twitch muscle fiber content in the convexity of the curvature in a subset of AIS subjects, suggesting HIF-2α dysregulation as a possible risk factor for AIS. The role of HIF-2α in paraspinal muscle function during spinal growth and its relevance in AIS prognosis warrants further investigation.

KEYWORDS
adolescent idiopathic scoliosis, hypoxia, hypoxia inducible factors, paraspinal muscle

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1 | INTRODUCTION

Adolescent idiopathic scoliosis (AIS) is the most common pediatric spinal deformity, affecting children between 11 and 18 years old with a prevalence rate value ~3.5%, with females having a higher risk of progression compared to males.[1,2] Management of AIS is particularly challenging due to difficulty in accurately and reliably prognosing progression. If left untreated, severe curves can lead to disfigurement, back pain, early degeneration, and may be detrimental to respiratory function.[3] We believe that understanding the etiology of AIS is important to the prediction and effective early control of curve progression.

To date, there is no ascertained theory for the pathogenesis of AIS. Genome Wide Association Studies (GWAS) have identified single nucleotide polymorphisms (SNPs) in multiple genetic loci associated with neuromuscular, skeletal, and connective tissue systems.[4–7] Although paraspinal muscles are not defined as part of the functional spinal unit, they are critical in stabilizing the spinal column, and changes in musculature are known to be associated with spinal degeneration and low back pain.[8] We previously reported unbalanced paraspinal muscle activities in progressive AIS patients in comparison with healthy subjects, implicating asymmetric dysregulation of musculature may be an underlying mechanism.[9] A related study on paraspinal muscle fibers in scoliotic patients reported a higher abundance of fast twitch, glycolytic type II b muscle fibers on both concave and convex sides of the spinal curvature, whereas a significant reduction of slow twitch type I muscle fiber was found on the concave side of the curve.[10] Asymmetric distribution of calmodulin, a protein that regulates Ca^{2+} transport for muscle cell contraction, has also been also reported, where a higher expression was found on the convex side of paraspinal muscle in AIS patients.[11] These findings indicate that abnormalities in paraspinal muscle generation and/or activity may have a role in the pathology and progression of AIS. However, it is not clear whether such changes are the primary causes of scoliosis, or secondary to the curvature itself.

Physiological responses under hypoxia are regulated by the alpha subunits of hypoxia inducible factors HIF-1α and HIF-2α. Under normoxia, HIF-αs are polyubiquitinated by an E3 ubiquitin ligase, von Hippel–Lindau protein (Vhl), resulting in proteosome dependent degradation. While in hypoxia, HIF-αs are stabilized and induced in the cell nuclei to activate gene transcription.[12] The physiological oxygen tension in skeletal muscle under substantial contraction is about 5% O_2. Myocytes are therefore considered experiencing a hypoxic microenvironment. Hypoxia could regulate muscle-resident stem cell (satellite cell) activity and muscle regeneration in response to injury.[13] In fact, alteration of skeletal muscle fiber types was observed in mountain hikers under prolonged exposure of hypoxia, suggesting an adaptive mechanism of muscle toning under oxygen tension.[14] In line with the finding, overt expression of HIF-1α was reported to promote the synthesis of fast twitch type II muscle fibers in adult rat muscles under stimulated contraction.[15] HIF-2α has been shown to induce slow twitch oxidative type I muscle fiber-related genes, including *MyoHCI*, *Myoglobin*, *Calmodulin2*, and *Troponin I*.[16] Building on these findings, we hypothesized that progressive AIS is linked to HIF-α dysregulation and therefore abnormal myogenesis in the paraspinal muscles. In the current study, we aimed to test if paraspinal muscle cells from progressive AIS subjects have different HIF-1α and HIF-2α protein expression and show different myogenic potential from non-scoliosis controls under hypoxia.

2 | MATERIALS AND METHODS

2.1 | Subjects recruitment

Ethics was approved by the local institutional review board. All methods were carried out in accordance with the Declaration of Helsinki principles.[17] The inclusion criteria were adolescents diagnosed with AIS and developed a Cobb angle $\theta > 45^\circ$ (representing progressive cases with severe curvature), pending deformity correction surgery (Table S1). Patients were excluded if Cobb angle was $<45^\circ$, having other pre-existing orthopedic or systemic conditions, or congenital spinal abnormalities. The non-scoliosis control group was drawn from ethnically matched subjects with degenerative disc disease. Low dose biplanar stereoradiography (EOS, EOS® Imaging, Paris, France) of patients in a standing position were obtained for Cobb angle measurement. Cobb angle was measured by two independent readers blinded to the patient details.[18] In brief, an average score of the Cobb angle was recorded if the measured degrees were within 5°. Any deviation beyond this and discrepancies with other measurements were decided by consensus between the same two readers.

2.2 | Cell culturing and determination of HIF-1α/HIF-2α protein expression

Paraspinal muscle specimens from severe AIS patients and non-scoliosis control subjects were harvested to establish myoblast cultures. For one of the AIS subjects developed multiple curves (AIS30), specimens from the upper and lower thoracic curves were separately harvested. Briefly, ~1 cm^3 muscle biopsy was harvested bilaterally from the superficial multifidus muscle at the apex in AIS or at the ends of the wounds for non-scoliosis control. Thereafter, it was minced into pieces (~1 mm^3) for digestion in 1:1 TrypLE Express (12604021, Thermo Fisher Scientific)/Dulbecco's Modified Eagle Medium High Glucose (DMEM-HG) (12100046, Thermo Fisher Scientific) at 37°C for 30 min. Subsequently, the tissue was further digested in 0.2% Collagenase type II (17101015, Gibco) in DMEM-HG at 37°C for 30 min. Primary myoblasts were subsequently cultured in DMEM-HG, 10% fetal bovine serum (FBS). Myogenic induction of myoblasts was induced in myogenic differentiation medium: DMEM-HG; 2% horse serum for 4 days under normoxia (21% O_2) vs hypoxia (1% O_2). Protein levels of HIF-1α and HIF-2α expression were validated by western blot analysis and quantified via 96-well Human HIF-1α (DYC1935-5, R & D Systems) and HIF-2α (DYC2997-5, R & D Systems) enzyme-linked immunosorbent assay (ELISA) kits accordingly. Relative log2 fold change (log2FC) of HIF-α protein expression

(1% O_2)/(21% O_2) between cell lysates of progressive AIS cases vs non-scoliosis subjects were calculated.

2.3 | Myogenesis of AIS paraspinal myoblasts and gene expression characterization

Primary paraspinal myoblasts from AIS patients and non-scoliosis controls were subjected to myogenic induction medium: DMEM-HG; 2% horse serum under 1% or 21% O_2 for 4 days. Total RNA was extracted by Qiagen RNAeasy Mini kit (74106, Qiagen) and reverse transcribed by PrimeScript™ RT (RR037B, Takara) according to manufacturer's protocol. Quantitative PCR (QPCR) was performed using Power SYBR Green PCR mix (4367659, Thermo Fisher Scientific) to detect perinatal muscle fiber associated genes: *MYH3*, *MYH8*; slow twitch muscle fiber associated genes: *MYH7*; fast twitch muscle fiber associated gene: *MYH4*; and myogenic regulatory factor associated genes: *MYF5*, *MYOG*; and normalized by *GAPDH* expressions. Relative log2FC of gene expression was determined as ratio of expression under hypoxia to normoxia at Day 4. Analyzed genes and primer sequences are listed in Table S2.

2.4 | Histological analysis of slow and fast twitch paraspinal muscle fibers in AIS patients with HIF-α misexpression

To determine if HIF-α misexpression influenced the in situ slow and fast twitch paraspinal musculature, paraspinal muscle biopsy was obtained from the concave/convex apex of curvature in the AIS subjects and control subjects as aforementioned. Briefly, paraspinal muscle biopsy was fixed in 4% paraformaldehyde solution for overnight and placed in 70% ethanol at 4°C until parafilm embedding. Parafilm blocks were sectioned at 5 μm thickness. Eventually, paraffin sections were dewaxed in xylene, and rehydrated in a gradient of ethanol and PBS. Heat-mediated antigen retrieval was conducted at 95°C, 20 min, in citrate buffer, pH 6.0 (S236984-2, Dako). The muscle samples were subject to immunostaining with anti-slow twitch muscle related myosin heavy chain type I antibody (M8421, Sigma) 1:200 and anti-fast twitch muscle related myosin heavy chain type II antibody (M4276, Sigma) 1:200 for 4°C, overnight. Secondary antibody rabbit anti-mouse IgG Alexa 488 (A-11059, Thermo Fisher Scientific) was applied at dilution 1:500 for 2 h at room temperature. Finally, the sections were counter-stained with DAPI (H-1200, Vector laboratories). Whole slide scanning of images (40× magnification) was captured by Vectra Polaris (Perkinelmer) and images were exported to Image J software (version 1.53r) to measure the percentage of myosin heavy chain using threshold analyses.

2.5 | Statistical analysis

Data were analyzed and plotted using GraphPad Prism 8.0.1 (GraphPad Software Inc.), and all quantitative data are presented as the mean ± SEM. Statistical significance of gene and protein expression was tested by nonparametric Mann–Whitney *U* test.

3 | RESULTS

3.1 | Atypical HIF-2α suppression in myogenesis of paraspinal myoblast of AIS subjects under hypoxia

We first tested the expression pattern of HIF-αs in intervertebral disc (IVD) cells previously archived from AIS subjects and using cells from non-scoliotic disc degeneration subjects as control. Immunostaining showed similar HIF-1α and HIF-2α protein expression levels in non-scoliosis IVD cells cultured under normoxia or hypoxia for 3 days. This is in agreement with previous reports of oxygen independent HIF-αs expression in IVD cells.[19,20] Interestingly, we found a reduced expression of HIF-1α (Figure 1A) and HIF-2α (Figure 1B) in IVD cells from two-thirds of the AIS subjects and that the dysregulation of HIF-αs was associated with degree of hypoxia (1% O_2 vs 5% O_2). The findings therefore provided evidence of HIF-α misexpression in AIS subjects.

We further examined the HIF-1α and HIF-2α expressions in the paraspinal myoblasts obtained from the surgeries of AIS patients ($n = 18$) and non-scoliosis controls ($n = 5$). We cultured the myoblasts in myogenic differentiation media for 4 days and measured the protein expression of HIF-1α and HIF-2α under hypoxia vs normoxia (Figure S1). By ELISA quantitative analyses (Figure 2A), we observed that all non-scoliosis myoblasts exhibited ≥1.5 log2 fold induction of HIF-1α expression ($p = 0.0017$) in hypoxia vs normoxia [log2FC (hypoxia/normoxia)] and maintained HIF-2α expression with log2FC (hypoxia/normoxia) from −0.3 to 1.3. Western blot analyses validated the pattern of HIF-1α and HIF-2α expression (Figure 2B). By ELISA, we identified ~33% (6/18) AIS subjects exhibiting a suppression of HIF-2α expression with log2FC (hypoxia/normoxia) ≤ −1 in hypoxic cultured myoblasts (Figure 3B), whereas HIF-1α was induced with log2FC (hypoxia/normoxia) ≥ 1.5 (Figure 3A) in all tested AIS subjects. The atypical HIF-2α expression in hypoxic cultured AIS myoblasts was confirmed by western blots (Figure 3C). In summary, a suppressed HIF-2α expression was evidenced in myogenesis of AIS paraspinal myoblasts under hypoxia.

3.2 | Reduction of perinatal and slow twitch muscle fiber-associated genes in HIF-2α misexpressed AIS paraspinal myoblasts

To examine how myogenesis was affected under the HIF-2α dysregulation, we evaluated the gene expression of myogenic regulatory factors and perinatal/slow/fast twitch muscle fiber associated genes in the AIS and non-scoliosis paraspinal myoblasts under myogenic induction in hypoxic condition. With normalization of relative log2FC changes to Day 4 hypoxia/normoxia, expression of perinatal muscle

FIGURE 1 Protein expression of HIF-αs in IVD cells from AIS subjects and non-scoliosis control. By immunostaining (A) HIF-1α and (B) HIF-2α expressions were detected in non-scoliosis IVD cells cultured at different oxygen tensions for 3 days. HIF-1α expression was reduced in IVD cells from two different AIS patients: AIS(b)—16 years old female and AIS(c)—14 years old female, whereas HIF-2α expression was reduced in IVD cells from AIS patients: AIS(a)—17 years old male and AIS(c) in 1% O_2 (highlight in white asterisks). Non-scoliosis IVD cells were harvested from a 30 years old female healthy subject. DAPI was used for staining cell nuclei; Phalloidin was used for staining f-actin. Scale bars: 50 μm

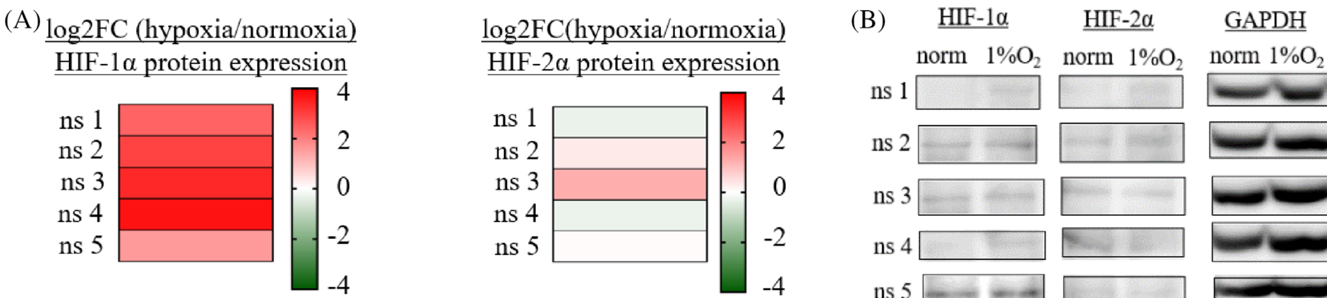

FIGURE 2 Protein expression of HIF-αs in paraspinal myoblasts from non-scoliosis controls. (A) ELISA protein quantification of HIF-1α and HIF-2α of myogenic-induced paraspinal myoblasts cultured in hypoxia (1%O_2) vs normoxia for 4 days. Relative log2FC (hypoxia/normoxia) of HIF-αs expression of paraspinal myoblasts under hypoxia/normoxia at day 4 was depicted in heatmap. (B) Western blot data indicated the induction of HIF-1α and sustained HIF-2α protein expression in paraspinal myoblasts of non-scoliosis controls: ns1, ns2, ns3, ns4, and ns5

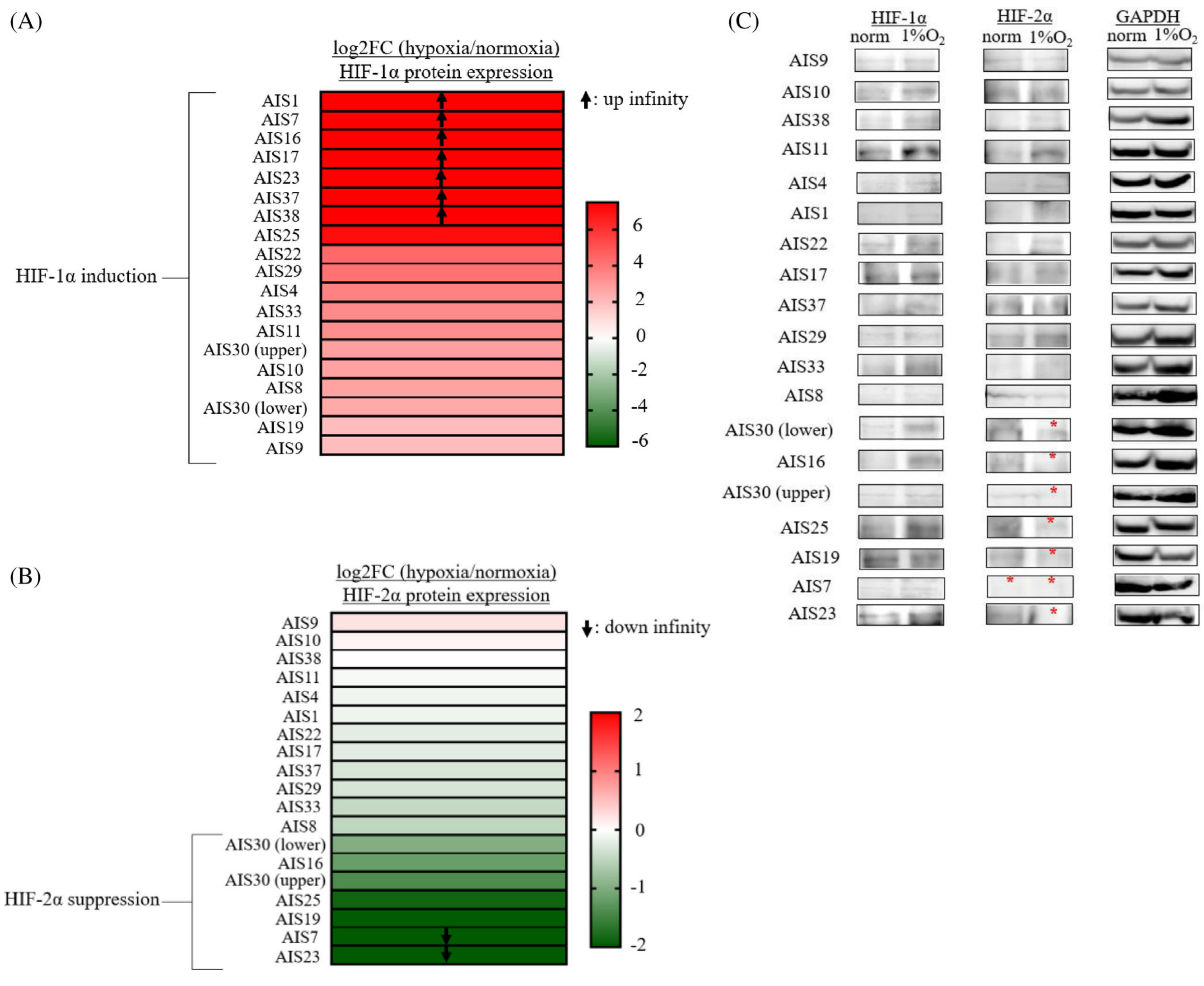

FIGURE 3 Differences of HIF-αs protein expression in paraspinal myoblasts from AIS subjects. ELISA protein quantification of (A) HIF-1α and (B) HIF-2α protein expression in myogenic-induced AIS paraspinal myoblasts hypoxia (1%O_2) vs normoxia for 4 days. Relative log2FC (hypoxia/normoxia) of HIF-αs expression of paraspinal myoblasts under hypoxia/normoxia at day 4 was depicted in heatmap. Up infinity: no HIF-1α expression was detected in normoxia, whereas HIF-1α was induced in hypoxia; down infinity: HIF-2α expression was detected or not in normoxia, whereas no HIF-2α expression was detected in hypoxia. (C) Western blot analyses shown HIF-1α induction in all hypoxic cultured AIS paraspinal myoblasts, whereas HIF-2α suppression in 6/18 AIS subjects: AIS7, AIS16, AIS19, AIS23, AIS25, and AIS30 (Table S1). HIF-2α suppression was indicated in red asterisks

fiber associated genes *MYH3* (MyHC emb), *MYH8* (MyHC neo) and slow twitch muscle fiber associated genes *MYH7* (MyHC I) were reduced in the myogenic induced paraspinal myoblasts derived from the HIF-2α misexpressed AIS subjects when compared to those from the normal HIF-2α expressed AIS subjects (Figure 4A,B,E). In contrast, expression of fast twitch muscle fiber associated gene *MYH4* (MyHC IIb) was similar among AIS subjects with normal and dysregulated HIF-2α expression (Figure 4C,E). No significant changes in expression of myogenic regulatory factor genes *MYF5* (proliferation and early differentiation factor) and *MYOG* (late differentiation and fusion factor) were found among the AIS subjects (Figure 4D). Altogether, these findings suggested a reduced expression of perinatal and slow twitch muscle fiber-associated genes, particularly in the AIS paraspinal myoblasts exhibiting HIF-2α misexpression under hypoxia.

3.3 | Reduction of slow twitch paraspinal muscle fiber at the convex paraspinal musculature in HIF-2α misexpressed AIS subjects

Given that AIS involves paraspinal muscle imbalance and slow/fast twitch muscle asymmetry,[9–11] and HIF-2α is important for regulation of slow twitch muscle fiber,[16] we asked if AIS patients with the HIF-2α misexpression exhibits a pattern of asymmetry in paraspinal muscle composition in situ. By immunostaining and cross-sectional area (CSA)

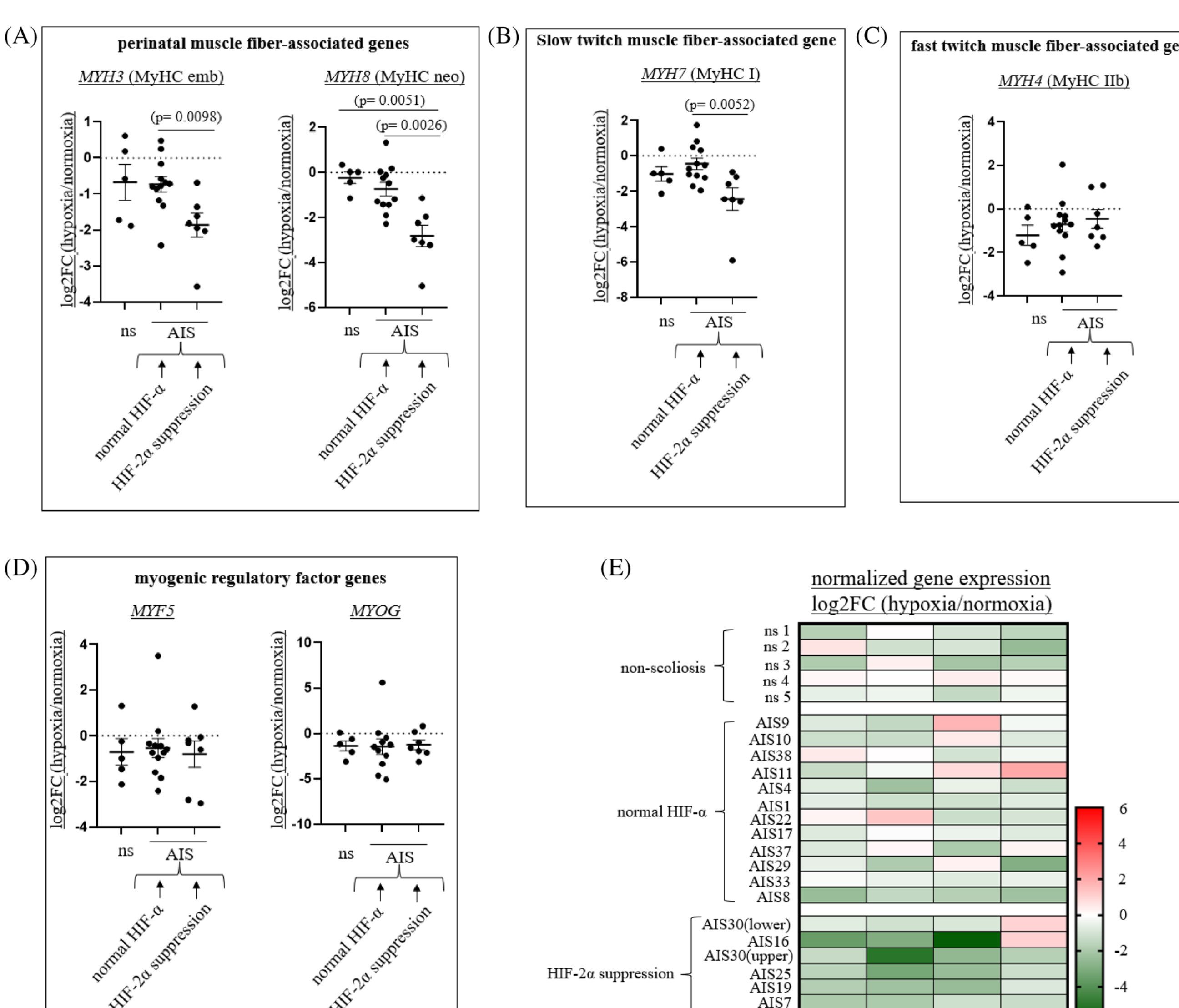

FIGURE 4 Gene expressions of perinatal/slow/fast twitch muscle fiber-associated genes and myogenic regulatory factor genes in in myogenic-induced HIF-2α suppressed AIS, HIF-α normal expressed AIS and non-scoliosis paraspinal myoblasts under hypoxia. Relative gene expression of (A) perinatal muscle fiber-associated genes: *MYH3*, *MYH8*; (B) slow twitch muscle fiber-associated genes: *MYH7*; (C) fast twitch muscle fiber-associated gene: *MYH4*; (D) myogenic regulatory factors: *MYF5*, *MYOG* was evaluated by log2FC (hypoxia/normoxia) at day 4 myogenesis of paraspinal muscle cells from AIS and non-scoliosis control subjects. Data show in mean ± SEM. Statistical significance was tested by nonparametric Mann–Whitney *U* test. (E) Heatmap depicted the differential changes of perinatal/slow/fast twitch muscle fiber-associated genes within individual of HIF-2α suppressed AIS vs HIF-α normal expressed AIS and non-scoliosis subjects

of fiber type quantification (Figure 5 and Figure S2), we detected a reduction of slow twitch myosin heavy chain I expression in the convex paraspinal muscles when compared to the concave counterpart in four of HIF-2α misexpressed AIS subjects ($p < 0.0001$) (Figure 5D and Figure S2), whereas no significant difference in five of the normal HIF-2α expressed AIS subjects (Figure 5B and Figure S2). In contrast, no significant difference in fast twitch myosin heavy chain II expression was detected between convex and concave paraspinal muscles in AIS subjects with normal or misexpressed HIF-2α. (Figure 5A,C and Figure S2) This suggests an asymmetrical paraspinal musculature in HIF-2α misexpressed AIS subjects, with a relative low proportion of slow twitch muscle fiber on convex side of the curvature.

4 | DISCUSSION

In this study, we first used IVD cells, which were derived from previous surgeries, as a pilot test to explore the hypothesis. It is important to note that the IVD cellular content is likely different with a higher proportion of notochordal cells in the adolescent AIS discs and more fibroblastic and senescent cells in degenerative discs. Additionally, AIS and degenerative discs may exhibit different intradiscal pressure as well as different inflammatory and nutritional condition. All of these might impact the disc cell behaviors including their responses to hypoxic stress. Nevertheless, we detected a suppression of HIF-1α and HIF-2α expression in IVD cells from two-

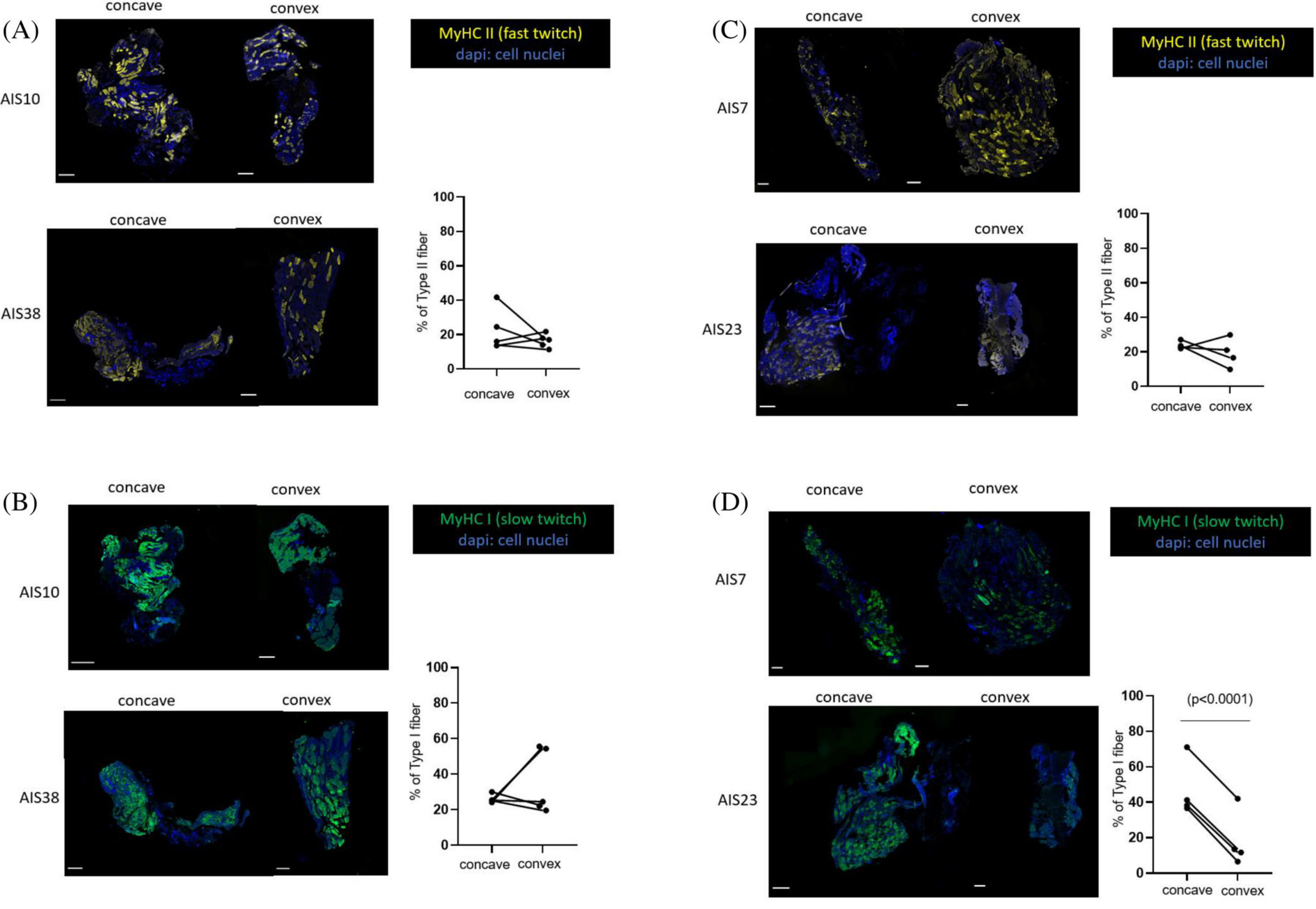

FIGURE 5 Asymmetry of slow twitch paraspinal muscle fiber type in HIF-2α misexpressed AIS subjects. Immunostaining showing fast twitch myosin heavy chain type II (MyHC II) expression and slow twitch myosin heavy chain type I (MyHC I) in concave/convex paraspinal muscle biopsy from normal HIF-2α expressed AIS subjects (A, B) and HIF-2α misexpressed AIS subjects (C, D). Scale bars: 250 μm. Relative % of cross section area (CSA) of fast twitch muscle fiber type II (MyHC II) and slow twitch muscle fiber type I (MyHC I) on both concave and convex side of the apex of the curve was calculated. Paired sample *t*-test was conducted for HIF-2α misexpressed AIS subjects: AIS7, AIS19, AIS23, AIS30 and normal HIF-2α expressed AIS subjects: AIS1, AIS4, AIS10, AIS11, AIS38

thirds of AIS subjects cultured under hypoxia. Thereafter, we expanded the investigation by recruiting a new cohort to harvest and study the paraspinal muscles. By further screening a cohort of progressive cases of AIS for HIF-αs dysregulation, we discovered a suppression of HIF-2α expression in ~33% (6/18) AIS paraspinal myoblasts and an induction of HIF-1α expression in all AIS subjects. AIS is a multifactorial disease and the fact that deviations in HIF-2α induction under hypoxia was found only in a subset of patients, suggested other non-HIF αs related causes in AIS development. Hence, the dysregulation of HIF-2α is likely one of the possible risk factors for AIS. Myogenic induction study identified a trend of reduced expression of perinatal and slow twitch muscle fiber-associated genes in HIF-2α misexpressed AIS paraspinal myoblasts. Furthermore, we found a lower proportion of slow twitch type I paraspinal muscle fibers in the convex side of curvature versus the concave side in the HIF-2α misexpressed AIS patients, whereas no significant difference in fast twitch type II paraspinal muscle fibers. Taken together, our findings indicated that a subset of AIS subjects exhibited a dysregulation of HIF-2α under hypoxic stress along with an asymmetric production of slow twitch muscle fibers, and therefore muscle tonicity in the paraspinal muscles at the apex of the curvature.

In addition to vertebrae, facet cartilaginous joint, tendons and ligaments, paraspinal muscle is considered as another primary structural component that influences the alignment of spinal column. Paraspinal muscles are the extensor muscles posterior to the vertebral bodies. Previous studies reported an unbalanced paraspinal muscle activity[9] and asymmetric distribution of fast twitch and slow twitch paraspinal muscle fiber between concave and convex side of curvature[10,11] in idiopathic scoliosis patients. Satellite cells (resident muscle stem cells), which are localized between the sarcolemma and the basal membrane of muscle fibers, play an essential role in synthesis of multinucleated myofibers during skeletal muscle development or in response to muscle injury. Satellite cells self-renewal and differentiation are regulated by oxygen tension. Of note, HIF-2α is the pivotal mediator of hypoxia signaling in the homeostatic maintenance of satellite cells.[13,21] While HIF-1α was reported to promote fast twitch muscle fiber formation, HIF-2α was shown to induce slow twitch muscle fiber.[15,16] Perinatal muscle fiber isoforms: MYH3 and MYH8, are not normally expressed

in adult muscle, except they are transiently re-expressed for skeletal muscle regeneration following injury. Previous studies reported a transition of perinatal muscle fiber to slow and fast twitch muscle fiber in myogenesis.[22] Our findings in the AIS subjects further support the regulatory role of HIF-2α in slow twitch muscle fiber maintenance may be due to the induction of perinatal muscle fiber isoforms. The finding of disproportionate slow twitch muscle fiber content in HIF-2α misexpressed AIS paraspinal musculature is intriguing. Slow twitch muscle fibers are related to endurance in long distance exercise. It is plausible that the weakness of slow twitch paraspinal muscle fiber activity on one side (the convex side) could cause or aggravate the curvature of the spinal column. How HIF-2α misexpression contributes to the asymmetric distribution of slow twitch muscle content in the spinal curvature requires further study. In previous studies, percentage of centralized nuclei of paraspinal myofibers in AIS was reported more than double of healthy skeletal muscle, indicating an active myofiber remodeling in AIS paraspinal muscles. However, the levels of remodeling were far below those reported for primary muscle disorders, mouse models of traumatic brain injury or Duchenne muscular dystrophy.[23] It is possible that the reduced slow twitch muscle fiber formation is caused by a suppression of HIF-2α activity, and that the altered muscle usage, unmet metabolic demands or abnormal mechanical loading on the convex side may reinforce further myogenic dysregulation. In consequence, an asymmetrical growth of musculature on the convex and concave side leads to spinal deformation. On the other hand, the paraspinal muscle samples for immunostaining of fast and slow twitch muscle fiber were harvested on the convex sides of AIS subjects identified with HIF-2α misexpression or normal HIF-α expression. Hence, the HIF-2α misexpression may be coupled to the curve-sidedness. Notwithstanding, we could not exclude any impact of left or right paraspinal muscle use in daily life on curvature formation.[24] A correlation study of left/right handedness with HIF-2α misexpression would help in addressing if curve-sidedness has an impact on paraspinal musculature and scoliosis configuration in the future.

There are a few limitations in this study. The sampling of paraspinal muscle, including the depth of dissection, the identification of muscle group (longissimus or iliocostalis or multifidus), and distance from the apex, may vary among the subjects due to differences in curvature. Moreover, the non-scoliosis controls were not age-matched. Use of age-matched post-mortem controls in future could enable a more precise comparison. The sedation level in the correction surgery of each AIS subject might be different and therefore induce different levels of in situ hypoxic stress and paraspinal muscle activity. In this regard, we studied the primary culture of paraspinal myoblasts under a constant level of hypoxia and differences between in vitro and in vivo hypoxic conditions may exist. Finally, physical activities, body mass index, gender, and household environment (high altitude vs sea level) could have unknown impacts on the hypoxic stress and muscle development in individual AIS subjects and therefore affect the observations.

In conclusion, the present study discovered an association of HIF-2α misexpression with slow twitch muscle dysregulation in AIS patients. Furthermore, a reduction of slow twitch muscle fiber was evidenced in the convex paraspinal musculature of HIF-2α misexpressed AIS patients. Our findings implicate a dysregulated response of hypoxic stress in paraspinal muscle and its potential contribution to spinal curvature. The cause of paraspinal muscular asymmetry in response to HIF-2α dysregulation under hypoxic stress warrants further investigations. AIS is a complex genetic disorder and the heritability of scoliosis is likely a combination of equivocal extrinsic environmental factors and intrinsic genetic factors. Impaired hypoxic response in paraspinal musculature may influence the muscle mechanics exerted on bone growth leading to increased susceptibility of scoliosis during adolescent skeletal growth spurt. In the future, it would be interesting to identify if AIS subjects with HIF-2α misexpression at early stage may have a higher penetrance rate of severe curves. This may indicate whether HIF-2α misexpression in paraspinal muscle is a causative factor of the deformity or secondary to the curvature itself. Nevertheless, such HIF-2α abnormalities in other structural elements of the spine may also have a role in the etiology of AIS. This study provides valuable insights into gene-environmental interactions in the pathogenesis of AIS, and the possibility of using HIF-2α as a biomarker in the prognosis of AIS and its progression.

AUTHOR CONTRIBUTIONS

Wai Kit Tam designed the project and conducted all cellular, gene and protein expression studies, analyzed data, and wrote the manuscript. Jason P. Y. Cheung, Paul A. Koljonen, Kenny Y. H. Kwan conducted the clinical assessment and provided human paraspinal musculature biopsies of AIS patients. Kenneth M. C. Cheung conceived the project, conducted the clinical assessment and provided human paraspinal musculature biopsies of AIS patients. Victor Y. L. Leung designed and supervised the project and interpretation of data. All authors reviewed and approved the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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