Deletion of narfl leads to increased oxidative stress mediated abnormal angiogenesis and digestive organ defects in zebrafish

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A B S T R A C T

Nuclear prelamin A recognition factor-like (NARFL) is a human protein that participates in cytosolic iron-sulfur (Fe–S) protein biogenesis and cellular defense against oxidative stress. Previous studies of Narfl knockout mice did not reveal well the regulatory mechanisms of embryonic development mediated by Narfl because the homologous mice die in utero. Here, we investigated the function of narfl in an established zebrafish knockout model by taking advantage of zebrafish external fertilization and ease of embryonic development examination. Our experiments showed that narfl deletion resulted in larvae lethality, subintestinal vessel (SIV) malformation and digestive organ defects in the early stages of embryonic development. Biochemical analyses and western blot revealed increased oxidative stress and upregulated hypoxia-inducible factor-1α (HIF-1α) expression in narfl−/− fish. The use of HIF-1α inhibitor 2-methoxyestradiol (2ME2) for the treatment of mutants partially rescued the SIV sprouting. These results suggest that narfl deletion causes increased oxidative stress and subintestinal vessel malformation, which further influence the development of digestive organs and might contribute to the lethality of the narfl knockout fish.

1. Introduction

Nuclear prelamin A recognition factor-like (NARFL), also known as cytosolic iron-sulfur-folding assembly component 3 (CIA03) or iron-only hydrogenase-like protein 1 (IOP1), is a human protein that is homologous to Nar1. Nar1 is an essential component of the cytosolic iron-sulfur (Fe–S) cluster assembly (CIA) pathway in yeast. Fe–S proteins containing [2Fe–2S] and [4Fe–4S] [1,2] participate in many biologic processes, including Krebs cycle reactions, oxidative phosphorylation, translation, iron regulatory pathways and purine metabolism [3]. Previous studies showed that NARFL functions in the cytosolic Fe–S protein biogenesis and knockdown of NARFL causes decreased activity of cytosolic aconitase, an Fe–S protein in mammalian cells [4]. In yeast, genetic deletion of Nar1 is fatal and results in impairment of the cytosolic Fe–S protein assembly [5]. In mice, Iop1/Narfl knockout yields intrauterine death and Iop1 loss in mouse embryonic fibroblasts leads to lethality [6].

It was reported that NARFL knockdown in mammalian cells causes increased levels of hypoxia-inducible factor-1α (HIF-1α) protein under normoxic and hypoxic conditions [7], and recent study has shown that NARFL is a key element in cellular defense against oxidative stress [8]. In our previous work, NARFL (Ser161Ile) was identified as a causative gene in a family with diffuse pulmonary arteriovenous malformations (dPAVMs), in which the proband died of pulmonary hypertension at a young age, and we observed abnormal development of the subintestinal vessels using a narfl-knockout zebrafish model [9]. Previous studies provide insights regarding the interaction between oxidative stress, angiogenesis and vascular diseases, indicating that oxidative stress plays a positive role during angiogenesis [10–13]. The anti-angiogenic effects of antioxidants also reveal the effects of oxidative stress on angiogenesis and related developmental processes [14–17]. Patricia Siquest et al. have summarized that oxidative stress (mainly reactive oxygen species, ROS) participates in the pulmonary vasculature’s responses under hypobaric hypoxia, and pointing out that HIF-1α directly induces the activation of NADPH oxidase and contributes to ROS production [18]. In addition, it was reported that oxidative stress contributes to the inactivation of prolyl hydroxylase 2 (PHD2) and results in the stabilization and activation of HIF-1α [19]. These results suggest that ROS and HIF-1α signals are interconnected in vascular development and remodeling, both of which are related to the role of NARFL.
Therefore, we proposed the present study to explore the function of narfl using an established zebrafish model. We observed that all narfl-knockout progenies died at 9–18 days post-fertilization (dpf). Mutants demonstrated severe defective development of the subintestinal vessels and digestive organs, increased oxidative stress, upregulated HIF-1α and angiogenesis linked gene expressions. Furthermore, with the treatment of 2-methoxyestradiol (2ME2), an inhibitor of HIF-1α, the subintestinal vessel (SIV) sprouting could be partially rescued in narfl+/− fish. Our study provides new insight into the understanding of narfl function in oxidative stress and HIF-1α induced angiogenesis from a developmental perspective.

2. Materials and methods

2.1. Zebrafish maintenance

The wild type zebrafish was maintained in our laboratory under standard conditions [20]. Zebrafish embryos developmental stages were determined by hour post-fertilization (hpf) or dpf [21]. All experiments were performed following the National Guide for the Care and Use of Laboratory Animals and the study was approved by the Institute of Hydrobiology, Chinese Academy of Sciences (Approval ID: IHB 2013724).

2.2. Generation of narfl mutant lines and RNA injection

Narfl knockout was performed using a CRISPR/Cas9 system as previously described [9]. Homozygous mutants were obtained from F1 embryos that were derived from F0 and wild type intercross. Two mutant lines, namely narfl+/− and narfl−/−, were genotype-identified using primers shown in Table S1 in supplementary information. For narfl mRNA injection, full length of zebrafish narfl cDNA was cloned into pSP64-poly A vector and synthesized into the capped mRNA using a mMESSAGE mMACHINE SP6 Kit (Ambion, Austin, TX, USA). About 300 ng/μL narfl mRNA was injected into 1–2 cell stage embryos.

2.3. Reverse transcription PCR (RT-PCR) and quantitative real time PCR (qPCR)

Total RNA was extracted from the embryos at different embryonic stages and adult tissues using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). The cDNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA) following the standard instructions. Transcript levels of narfl at different embryonic stages and adult tissues in wild type fish were analyzed using RT-PCR. The qPCR was performed using the AceQ qPCR SYBR Green Master Mix (Vazyme, Nanjing, China). Data were analyzed using a ΔΔCt method and β-actin 1 served as the house-keeping gene. All the experiments were performed in triplicate and the primers are listed in Supplementary Table S1.

2.4. RNA-seq analysis

Total RNA was isolated from 4 dpf narfl+/+ and narfl−/− larvae using the Trizol reagent following the standard protocols. About 100 bp paired-end reads were obtained by sequencing on the Illumina (HiSeq 2500/4000) platform. After removing adaptor sequences and low-quality sequences, the remaining paired-end clean reads were aligned to zebrafish genome using TopHat v2.0.12. Normalized gene expression level was separately calculated as Fragments per Kilobase of transcript per Million fragments mapped (FPKM). The cutoff value was determined based on a 95% confidence interval for all the FPKM values to evaluate gene transcriptional activity.

2.5. Whole-mount in situ hybridization

Whole-mount in situ hybridization of anti-sense RNA probes, including narfl, fabp1a (liver probe), fabp2 (intestine probe), ela2l (exocrine pancreas probe) and hif1ab, was performed as previously described [22–24].

2.6. Alkaline phosphatase staining and confocal imaging

Embryos were fixed overnight in 4% paraformaldehyde and processed for alkaline phosphatase staining as previously described [25]. The fl:GFP/narfl−/− homozygous lines were generated by mating the fl:GFP/narfl+/− adult fish, which were obtained by hybridizing the fl:GFP/Con and narfl+/− fish. Confocal images of 3 dpf transgenic embryos were generated using a Zeiss ISM 710 confocal microscope.

2.7. Western blot

Protein extracts were subjected to 8%–12% SDS-PAGE and transferred to the PVDF membrane (Millipore, Hayward, CA, USA). Rabbit anti-Narfl (1:1000, Abcolon, Wuhan, China), rabbit anti-HIF-1α (1:500, Boster, Wuhan, China), rabbit anti-Akt (1:1000, Cell Signaling Technology, MA, USA), rabbit anti-phosphorylated Akt (1:1000, Cell Signaling Technology, MA, USA), mouse anti-β-Actin (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used as the primary antibodies. The secondary antibodies conjugated to horseradish peroxidase were used at 1:5000 dilutions (proteintech, Wuhan, China).

2.8. ROS assay

Caudal fins of embryos were cut for genomic DNA isolation and genotyping using the NaOH lysis method as previously described [26]. After the fish tail genotyping, the rest of the body was sampled for ROS assay based on an established method [27]. Briefly, embryo was digested with 100 μL of 0.25% (w/v) trypsin/EDTA solution for 10 min before 200 μL of DMEM containing 10% (v/v) fetal bovine serum (FBS) was added to stop the reaction. Then sample was centrifuged at 2500 rpm for 5 min at 4°C to remove the supernatant, and cell pellet was washed in 200 μL of PBS containing 2% (v/v) FBS. After centrifugation again, cells were resuspended in 200 μL of PBS containing 2% FBS with 10 μM DCFH-DA probe (Beyotime, Nanjing, China) and incubated at 37°C for 30 min. Sample without probe incubation was served as a negative control. Fluorescence was detected at the excitation/emission wavelength of 488/525 nm using a FACSCanto Flow Cytometer (BD Bioscience, USA). To inhibit the ROS level, 1 mM N-acetylcysteine [27] (NAC, Sigma-Aldrich, St. Louis, MO, USA) was used for antioxidant exposure from 24 hpf, then 3 dpf embryos were collected for ROS measurement after two days of treatment.

2.9. Biochemical analyses

Cytosolic aconitase activity was determined using an Aconitase Activity Assay Kit (Sigma-Aldrich, St. Louis, MO, USA). Cellular redox substances including superoxide dismutase (SOD), glutathione sulfur transferase (GST), glutathione (GSH), and malondialdehyde (MDA) were detected by colorimetry using commercially available assay kits (Beyotime, Nanjing, China). All assays were implemented with three replications.

2.10. Drug exposure

Embryos were exposed to drug at 24 hpf before the first sprouts form the first subintestinal venous plexus vessel [28]. Because the endogenous alkaline phosphatase activity reaches a relatively high level at 3 dpf, and the subintestinal venous plexus starts regressing at 4 dpf, thus 3 dpf embryos were collected for subsequent testing after two days.
of treatment. Embryos were dechorionated at 24 hpf and distributed on a 6-well plate (20 embryos per well) for drug exposure. The 2ME2 (Selleck, Houston, TX, USA) used in the experimental group was 0.2 μM or 2 μM and the control group was treated with dimethylsulfoxide (DMSO) at the same concentration used in 2ME2 treated fish (See the Supplementary Fig. S1 for drug concentration selection).

2.1. Statistical analyses

Student’s t tests were used for statistical analyses with SPSS 20.0 (SPSS Inc., Chicago, USA). All data were expressed as the mean and standard deviation (mean ± SD). Results were considered significant when the probability of making a type I error was less than 5% (P < 0.05).

3. Results

3.1. Expression patterns of narfl in wild type fish and generation of narfl mutant lines

RT-PCR results showed that narfl was widely expressed in the early stages of embryonic development (Fig. 1A) and adult tissues, including brain, gill, eye, heart, liver, spleen, muscle, kidney and intestine (Fig. 1B). The spatio-temporal expression patterns provided by whole-mount in situ hybridization showed that narfl was expressed in liver and intestine at 3 dpf and the expression increased over time (Fig. 1C–H).

CRISPR/Cas9-mediated narfl knockout was described in our previous work [9]. Two independent mutant lines, respectively containing 4 bp insertion and 5 bp deletion plus 4 bp insertion in the target region, were obtained and confirmed by sequencing (Fig. 1I). Compared with the wild type narfl that encoded a protein of 477 amino acids, the putative translation products with premature stop codons of the two mutant lines contained 198 and 221 amino acid residues, respectively. The cytosolic aconitase activity was diminished in narfl−/− fish compared with the control siblings (Fig. 1J). Western blot revealed obviously decreased NARFL protein levels in mutant lines (Fig. 1K-L). In addition, we examined the expressions of narfl and narfl associated genes such as ciao1, rsad2 and ciao2a, and the qPCR results are presented in Supplementary Fig. S2.

3.2. Larvae lethality and subintestinal vessel malformation in narfl−/− fish

The narfl knockout resulted in zebrafish larvae death from 9 to 18 dpf (Fig. 2A). Alkaline phosphatase staining of 3 dpf embryos showed maldevelopment of subintestinal vessels in narfl−/− fish (Fig. 2B–D). The confocal images of fl:GFP/narfl+/−offsprings revealed subintestinal...

![Fig. 1. Expression patterns of narfl in wild type fish and generation of narfl-knockout lines. A-B: RT-PCR evaluated the expression patterns of wild type zebrafish narfl gene at different embryonic stages (A) and in various adult tissues (B). C-H: Spatio-temporal expression pattern analysis of narfl with whole-mount in situ hybridization in wild type fish. C, E and G: Lateral view anterior to the left. D, F and H: Dorsal view anterior to the top. L: liver; I: intestine. I: DNA sequences of narfl+/+ and narfl−/− zebrafish. The narfl−/−-1 represented a mutant line containing 4 bp insertion and narfl−/−-2 represented a mutant line containing 5 bp deletion plus 4 bp insertion. J: Relative cytosolic aconitase activity measured in narfl+/+ and narfl−/− fish. K-L: Western blot analysis of NARFL protein in mutants and control siblings. *, P < 0.05, ***, P < 0.001.](image-url)
vascular abnormal sprouting in fl:GFP/narfl−/− larvae (Fig. 2E-G). Wild type zebra narfl mRNA was synthesized and injected into 1–2 cell stage narfl−/− embryos and the defective phenotype of subintestinal vessels was partially rescued (Fig. 2H-I).

3.3. Disturbed signaling pathways including increased angiogenesis and glycolysis signals in narfl−/− fish

We performed transcriptome analysis of narfl+/+ and narfl−/− larvae at 4 dpf using RNA sequencing. The differentially expressed genes between wild type and mutant fish were highly involved in metabolic pathway and fat digestion and absorption pathway. Genes associated with glycolysis (e.g. hkdc1, aldoca, gck, pgk1 and slc2a1a) and angiogenesis (e.g. vegfaa, kdr, pdgfra, angptl1, tek, tgfβh2 and tgfbr2), especially genes in the Bmp pathway (e.g. bmp2b, bmp4, bmp7b and bmp16) were upregulated in narfl−/− fish. The qPCR results verified the increased expression of some of the above genes and other angiogenesis associated genes (vegflb and pdgfrb) chosen based on previous studies [29–36] (Supplementary Fig. S3). The fabp1b.1, fabp2 and apoa4hl.2 genes involved in fat digestion and absorption pathway, and ela2, ela2l and try genes involved in pancreatic secretion were downregulated in narfl−/− fish. Data are presented in Supplementary Table S2.

3.4. Maldevelopment of digestive organs in narfl−/− fish

Reduced food intake was observed in narfl−/− larvae compared with the control siblings (Fig. 3A). In situ hybridization of liver probe (fabp1a), intestine probe (fabp2), and exocrine pancreas probe (ela2l) at 4 dpf showed decreased signal expression in mutants (Fig. 3B–J), as confirmed by qPCR data (Fig. 3K). This was consistent with the downregulated genes involved in fat digestion and absorption as well as pancreatic secretion in the transcriptome data (Supplementary Table S2).

3.5. Increased oxidative stress and upregulated HIF-1α protein expression in narfl−/− fish

In narfl−/− fish, we observed significantly elevated ROS level (P < 0.001), which could be downregulated after NAC treatment, compared with the wild type fish (Fig. 4A–C). The cytosolic SOD activity and GST activity were diminished in narfl−/− fish (Fig. 4D-E). Cytosolic content of GSH was decreased, while the MDA content had the increasing tendency but was not significantly affected in mutants (Fig. 4F-G). The result of in situ hybridization of 4 dpf larvae showed increased expression of hif1ab in mutants (Fig. 4H). This was confirmed by western blot result, which revealed upregulated HIF-1α protein level in narfl−/− fish (Fig. 4I-J). In addition, we observed increased phosphorylated Akt (p-Akt) protein level in mutant fish, as determined by the increasing tendency but was not significantly elevated in mutants (2-OG), and undergoes proteasomal degradation finally [37,38]. Previous research demonstrated that the stabilization and activation of

4. Discussion

In the present study, the knockout of narfl in zebrafish resulted in decreased aconitase activity and larvae lethality. In narfl−/− fish, we observed defective development of subintestinal vessels, increased oxidative stress and HIF-1α expression. Using HIF-1α inhibitor 2ME2 for treatment partially rescued the subintestinal vascular sprouting in mutants, and suppressed the upregulated expressions of genes involved in angiogenesis and glycolysis signals. These results indicate that narfl knockout activated ROS-HIF-1α signal and mediated abnormal sprouting of subintestinal vessels, which led to digestive organ maldevelopment and probably larvae lethality.

The oxidative stress was increased and HIF-1α expression was upregulated in narfl knockout fish, which were consistent with the findings in mammalian cells [7,8]. In normoxic condition, HIF-1α is mainly hydroxylated by PHD2 in the presence of O2, Fe2+ and 2-oxoglutarate (2-OG), and undergoes proteasomal degradation finally [37,38]. Previous research demonstrated that the stabilization and activation of
HIF-1α were regulated by PHD2, an Fe(II)-dependent dioxygenase that could be inactivated by oxidative stress [19]. In our previous study, we found that NARFL expression was decreased and ferric iron was accumulated in the dPAVMs patient's lung tissue [9]. Therefore, it is conceivable that NARFL knockout causes abundant ferric iron but a deficiency of ferrous iron, and thus inactivates PHD2. Furthermore, ROS was reported to independently play a positive role in the upregulation of HIF-1α concentration under normal circumstances [39–42], which implies that increased ROS may directly activate HIF-1α in narfl−/− fish. In a previous study of gastrointestinal vascular malformation (GIVM), it was reported that abnormally high expression of HIF-1α and HIF-2α could cause GIVM through inducing angiogenesis and SIV sprouting in zebrafish, which is similar to the phenotype observed in our narfl−/− fish [43]. Referring to the angiogenesis during zebrafish embryo development, Vegf, Notch and Bmp signals were all reported to be involved in the subintestinal venous plexus development [28,44]. Vegf promoted the sprouting of the predominantly venous plexus and Bmp promoted the outgrowth of the structure, but whether Notch

Fig. 3. Zebrafish narfl deletion induced defective development of digestive organs. A: Food intake 30 min after 7 dpf larvae fed with egg yolk and 10 dpf larvae with fairy shrimp. Larvae were imaged using a dissection microscope. B-J: In situ hybridization of fabp1a (B-D), fabp2 (E-G), and ela2l (H-J) at 4 dpf. Red arrowheads indicate the liver, black arrowheads indicate the intestine and white arrowheads indicate the exocrine pancreas. For in situ hybridization results, at least 18 embryos per genotype were analyzed in two separated experiments. K: The qPCR result of fabp1a, fabp2 and ela2l genes in narfl−/− fish and control siblings. *, \( P < 0.05 \), **, \( P < 0.01 \). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
signal worked in this process remains controversial [28,44]. In addition, previous investigations of cultured rat sympathetic neurons showed that ROS was involved in BMP-induced dendritic growth [45]. In a study of fibroblastosis ossificans progressiva, HIF-1α was found to play a positive role in the hypoxic amplification of BMP signaling in the endosomial compartment of hypoxic connective tissue progenitor cells [46]. Therefore, we speculate whether ROS induced HIF-1α activation and mediated the SIV sprouting through angiogenesis linked pathways especially the Bmp signaling in narfl−/− fish. Correspondingly, both the RNA-seq and qPCR results showed that genes involved in Bmp pathway, including bmp2b, bmp7b and bmp16, were upregulated in mutant fish. Using 2ME2 for HIF-1α inhibition, the SIV sprouting in mutants was partially rescued and the increased expressions of Bmp-related and other angiogenesis linked genes were suppressed. These results suggest that Bmp signaling is dominantly involved in ROS-HIF-1α-induced subintestinal vessel malformation in zebrafish. Taken together, we conclude that knocking out zebrafish narfl causes increased oxidative stress, which activates Bmp signaling and results in SIV sprouting, as shown in the functional mechanism diagram of narfl in Fig. 6.

Zebrafish subintestinal plexus is a set of venous angiogenic vessels that absorb nutrients from the yolk and deliver them to the adjacent yolk syncytial layer, which later supports the distribution of blood to the digestive system [47,48]. This plexus comprises vessels that vascularize the gut, liver and pancreas, and is considered as an ideal model to investigate organ-specific vessel formation [44]. We performed a series of in situ hybridization using probes against the digestive organs, and noticed that the liver, intestine and exocrine pancreas were smaller in narfl−/− fish. Feeding experiments revealed that food intake in mutants was significantly less compared with the control siblings. All these information suggests that malformation of subintestinal vessels in narfl-knockout fish might retard the organ-specific vessel formation to impair the normal development of digestive organs.

In the present study, we utilized a narfl-knockout zebrafish model with the subintestinal vessel disorder and demonstrated that narfl was essential for embryonic development and organism defense against oxidative stress. We conclude that increased oxidative stress induced by narfl deletion disturbs the development of subintestinal vessels and digestive organs through the ROS-HIF-1α-Bmp pathway, and may contribute to the death of narfl−/− fish. In our previous study, NARFL was identified as a possible genetic basis underlying dPAVMs [9], and in the present study, narfl knockout led to an abnormal angiogenic phenotype in zebrafish. In fact, oxidative stress was reported to be
Fig. 5. The 2ME2 treatment rescued the subintestinal vessel defect. A-B: Alkaline phosphatase staining statistics for 3 dpf embryos treated with 0.2 μM 2ME2. Data are presented from three independent experiments in which at least 30 embryos per group were analyzed. C-M: The qPCR analyses of hkd1, aldoca, gck, bmp2b, bmp7b, bmp16, vegfab, kdr, pdgfrb, angpt1 and tek in narfl+/+ and narfl−/− fish after two days of 2 μM 2ME2 treatment. *, P < 0.05, **, P < 0.01, ***, P < 0.001.

Fig. 6. Hypothetical schema for narfl deletion-induced oxidative stress mediated abnormal angiogenesis in zebrafish. Solid lines indicate the pathways revealed by previous studies and our research, and dotted lines represent the hypothesis part. Dotted box indicates the proteosomal degradation process of HIF-1α.
closely associated with the development of pulmonary vascular disease and pulmonary hypertension [49–52]. Therefore, our observation of the abnormal angiogenesis mediated by increased oxidative stress in a narfl knockout zebrafish model provides evidence for a deeper understanding of the pathological mechanism of cytosolic iron-sulfur assembly component involved in vascular remodeling of pneumonia.

It is worth noting that both NAC and 2ME2 treatments failed to rescue the lethal phenotype caused by narfl knockout (data not shown). Possible speculations include: 1) in addition to the ROS signal, there might be other potential mechanisms through which narfl affects embryonic development; 2) the mode of drug administration could not properly balance the effects of ROS due to the inability to distinguish the harmful effects of excessive ROS and the physiological ROS-mediated processes [53,54]. Detailed functional studies of NARFL in other cytological and mammalian experimental systems seem necessary, and it would be challenging to investigate whether NARFL is responsible for any other developmental processes apart from our proposed regulatory network in the present study.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

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References

[1] R. Lill, Function and biogenesis of iron-sulfur proteins, Nature 460 (2009) 831.
[2] H. Ye, T.A. Rouault, Human Iron – Sulfur cluster Assembly, Cellular iron homeostasis, and disease, Biochemistry 49 (2010) 4945–4956.
[3] T.A. Rouault, W.-H. Tong, Iron-sulfur cluster biogenesis and mitochondrial iron homeostasis, Annu. Rev. Genet. 49 (2015) 37–58.
[4] D. Song, F.S. Lee, A role for IOP1 in mammalian cytosolic iron-sulfur protein biogenesis, J. Biol. Chem. 283 (2008) 9231–9238.
[5] J. Bøk, A.J. Pierric, D.J.A. Netz, U. Mühlendoeff, R. Lill, The hydrogenase-like Nar1p is essential for maturation of cytosolic and nuclear iron-sulfur proteins, EMBO J. 23 (2004) 2126–2138.
[6] D. Song, F.S. Lee, Mouse knock-out of IOP1 protein reveals its essential role in mammalian cytosolic iron-sulfur protein biogenesis, J. Biol. Chem. 286 (2011) 15797–15805.
[7] J. Huang, et al., IOP1, a novel hydrogenase-like protein that modulates hypoxia-inducible factor-1α activity, Biochem. J. 401 (2007) 341.
[8] M.V. Corbin, D.A.P. Rockx, A.B. Oostra, H. Hoenge, J.C. Dorsman, The iron-sulfur cluster assembly network component NARFL is a key element in the cellular defense against oxidative stress, Free Radic. Biol. Med. 89 (2015) 863–872.
[9] H.-Z. Liu, et al., A novel mutation in nuclear prelamin a recognition factor-like (NARFL) gene disrupts the metabolism of hemin in zebrafish, Dev. Dyn. 237 (2008) 2200–2205.
[10] Y.-W. Kim, T.V. Byzova, Reactive oxygen species in the control of hypoxia-inducible factor-mediated gene expression, Semin. Cell Dev. Biol. 16 (2005) 474–486.
[11] R.S. BelAlaa, et al., Redox-sensitive regulation of the HIF pathway under non-hypoxic conditions in pulmonary artery smooth muscle cells, Biomedical Research 38 2004.
[12] N. Gao, et al., Vanadate-induced expression of hypoxia-inducible factor 1α and vascular endothelial growth factor through PI3K/Akt signaling pathway and reactive oxygen species, J. Biol. Chem. 283 (2008) 18820–18826.
[13] D.E. Richard, F. Berra, J. Pouységur, Non-hypoxic pathway mediates the induction of hypoxia inducible factor alpha 1 and HIF-1α in vascular smooth muscle cells, J. Biol. Chem. 275 (35) (2000) 26765–26771, https://doi.org/10.1074/jbc.M603325200 PMID: 10837481.
[14] N. Feng, et al., HIF-1α and HIF-2α induced angiogenesis in gastrointestinal vascular remodeling and repression of thalidomide, Sci. Rep. 6 (2016) 27280.
[15] G. Hen, et al., Venous-derived angioblasts generate organ-specific vessels during zebrafish embryonic development, Development (Cambridge, England) 142 (2015) 4273–4286.
[16] V. Chandrasekaran, C. Lea, J.C. Sosa, D. Higgins, P.J. Lein, Reactive oxygen species are involved in BMP-induced denticles growth in cultured rat sympathetic neurons, Mol. Cell. Neurosci. 67 (2015) 116–125.
[17] H. Wang, et al., Cellular hypoxia promotes heterotopic ossification by amplifying BMP signaling, J. Bone Miner. Res. 31 (2016) 1652–1665.
[18] L. Carvalho, C.-P. Heisengen, The yolk syncytial layer in early zebrafish development, Trends Cell Biol. 20 (2010) 596–599.
[19] A. Donovan, et al., Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter, Nature 403 (2000) 776–781.
[20] E.A. Zemskov, et al., Biomechanical Forces and Oxidative Stress: Implications for Pulmonary Vascular Disease, Antioxidants & Redox Signaling, 2019.
[21] K. Brassington, S. Selemidis, S. Bozinovski, R. Vlahos, New frontiers in the treatment of comorbid cardiovascular disease in chronic obstructive pulmonary disease, Clin. Sci. 133 (2015) 389.
[22] S. Steven, et al., Pentamethyltetrazine in vivo treatment improves oxidative stress and vascular dysfunction by suppression of endothelin-1 signaling in monocytic-implicated pulmonary hypertension, PLoS One 8 (2013) e65792.
[23] V. Tseng, R.L. Sutilff, C.M. Hart, Redox Biology of Peroxisome Proliferator-Activated Receptorγ in Pulmonary Hypertension, Antioxidants & Redox Signaling, 2018.
[24] B. Poljak, S. Supat, I. Milisav, Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants, Oxidative Med. Cell. Longevity 2013 (2013) 566792–566792.
[25] A. van der Vliet, Y.M.W. Jansen-Heininger, V. Anathy, Oxidative stress in chronic lung disease: from mitochondrial dysfunction to dysregulated redox signaling, Mol. Asp. Med. 63 (2016) 59–69.