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Research Highlight

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Krüppel-like factor 1: a promising factor that promotes myocardial regeneration by triggering glycolytic shunt

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Recently, Ogawa *et al.* [1] confirmed for the first time that Krüppel-like factor 1(EKLF/KLF1) is a potent cardiomyogenic factor that regulates cardiomyocyte dedifferentiation and proliferation by triggering metabolic reprogramming. KLF1, a member of the C2H2 zinc-finger class, has a highly conserved DNA-binding domain and is a key transcription factor of erythropoiesis in mice and humans. KLF1 plays an important role in coordinating intracellular events during erythropoiesis, particularly in guiding megakaryocyte/erythrocyte progenitor cell lineage determination. KLF1 establishes the appropriate levels of morphologic and metabolic proteins and the terminal maturation for megakaryocyte/erythrocyte cell renewal and differentiation [2].

A prevous report found that the retinoic acid synthase RALDH2/ALDH1A2 is highly expressed in injured ventricles and facilitates myocardial regeneration [3]. However, KLF1 expression is incredibly upregulated when genetic attenuation of the retinoic acid signaling pathway reduces cardiomyocyte proliferation in injured zebrafish hearts, suggesting that KLF1 plays an essential role in cardiomyocyte proliferation and myocardial regeneration. Based on these facts, Ogawa *et al.* [1] investigated the novel function and mechanism of KLF1 in promoting cardiomyocyte proliferation and myocardial regeneration. Here, we provide a brief comment on KLF1, a promising transcription factor that promotes cardiac regeneration by triggering glycolysis shunt.

Ogawa *et al.* [1] demonstrated that enhanced KLF1 expression induces regenerative phenotypes in injured or uninjured zebrafish hearts. In the hearts with enhanced KLF1 expression, dedifferentiation markers Alcam and smooth muscle protein 22a (Sm22) all are upregulated. At the same time, mitotic phosphohistone $H3^+$ ($pHH3^+$) is upregulated in cardiomyocytes, resulting in a significant increase in the number of cardiomyocytes. Therefore, KLF1 can upregulate the expressions of genes related to cardiac dedifferentiation and proliferation, thereby promoting cardiomyocyte proliferation and myocardial regeneration.

Then, they explored the specific mechanism of cardiomyocyte proliferation and cardiac regeneration in the hearts with enhanced KLF1 expression. KLF1 affects myocardial regeneration through upstream and downstream sensitive cofactors. For instance, KLF1 regulates epigenetic and metabolic remodeling by upregulating genes related to myocardial development and downregulating genes related to sarcomere disassembly and muscle contraction. Reprogramming of these genes can improve cardiac function and reduce adverse ventricular remodeling upon myocardial infarction. Moreover, KLF1 up-regulates some proliferation genes like myocyte enhancer factor 2-positive proliferating cell nuclear antigen-positive ($Mef2^+PCNA^+$) and cyclin D1/2a that is D-type cyclin for myocardial cell G1-S commitment, thereby accelerating cardiomyocyte proliferation. Collectively, KLF1 can regulate the myocardial gene network to promote myocardial regeneration.

In addition, KLF1 can also promote cardiomyocyte proliferation and cardiac regeneration by regulating energy metabolism-related genes. A previous report found that KLF1 down-regulates the transcription of *PGC1α/PPARGC1A* gene [4] that controls mitochondrial biogenesis, maintenance and function, thus, reducing mitochondrial crest, ATP level and mitochondrial DNA content. Meanwhile, oxidative phosphorylation-related genes such as *atp5a1*, *cox6c* and *uqcrq* are downregulated by KLF1. However, is there any other pathway that provides sufficient energy for cardiomyocyte proliferation and cardiac regeneration after KLF1 down-regulates mitochondrial oxidative phosphorylation in cardiomyocyte? Interestingly, they found that when KLF1 down-regulates mitochondrial oxidative phosphorylation in cardiomyocytes, the concentrations of glucose and lactic acid in cardiomyocytes are decreased. Nevertheless, the pentose phosphate pathway (PPP) and serine synthesis pathway (SSP), which belong to glycolytic shunt, are significantly upregulated in proliferating cardiomyocytes, accompanied by a large amount of NADPH. These pieces of evidence showed that PPP and SSP provide energy for

cardiomyocyte proliferation after KLF1 downregulates mitochondrial oxidative phosphorylation. PPP, a branch of glycolysis, is the main source of NADPH required for ribonucleotide synthesis. Different modes of PPP can affect the glucose flow in glycolysis and *vice versa* [5]. Serine, as the non-essential amino acid, is critical for the growth and survival of proliferating cells, which supports several metabolic processes and contributes to the production of NADPH for antioxidant defense [6]. Switching from oxidative phosphorylation to glycolysis is very reminiscent of the Warburg effect.

The Warburg effect, which most commonly occurs in tumor cells, also regulates myocardial regeneration [7]. The metabolic transformation of the Warburg effect can provide more energy for cell proliferation and reduce the mitochondrial oxygen consumption rate, implying that the aerobic glycolysis pathway may provide sufficient energy for cardiomyocyte proliferation after KLF1 downregulates mitochondrial oxidative phosphorylation in cardiomyocytes. Furthermore, Warburg effect also exists in various non-tumor cells and plays an important role in non-tumor diseases. Our group explored the involvement of Warburg effect in the occurrence and progression of non-tumor diseases and found that the Warburg effect occurs in the proliferation of human aortic vascular smooth muscle cells (HA-VSMCs) induced by apelin-13 [8,9]. The Warburg effect is accompanied by a decrease in oxidative phosphorylation as well as an increase in glucose consumption and lactate production [8]. However, gene expression profiling and metabolomic analysis of KLF1-ON heart showed that glycolysis and tricarboxylic acid pathways are downregulated. The way of energy metabolism in KLF1-ON heart is inconsistent with the Warburg effect. In summary, the energy metabolism in KLF1-ON heart is transferred from mitochondrial oxidative respiration to glycolysis shunt (PPP and SSP), thereby reconnecting to cellular respiration and providing energy for cardiomyocyte proliferation and cardiac regeneration.

The above evidence suggests that KLF1 induces glycolytic shunt toward PPP and SSP to promote cardiomyocyte proliferation. KLF1-ON zebrafish heart increases the proliferative capacity, but decreases the structure related to scalability, resulting in heart failure symptoms and low survival rate. To avoid the occurrence of pathological characteristics of KLF1-ON zebrafish heart, Ogawa *et al.* [1] controled the transient activation of KLF1, which resulted in a significant increase in cardiomyocyte proliferation phenotype and ventricular volume without pathological phenomenon. So how does the injured heart regulate the KLF1 activation in physiological myocardial regeneration rather than pathologic cardiomyocyte dilation or fibrosis? Therefore, the role of KLF1 in cardiomyocyte proliferation and cardiac regeneration is very interesting and worthy of further exploration.

In the future, the combination of KLF1 and fluorescent protein using lineage tracking can more accurately and dynamically elucidate the myocardial regeneration process of zebrafish heart. Generally speaking, KLF1, as a transcription factor, promotes cardiomyocyte renewal in adult zebrafish heart by directing the

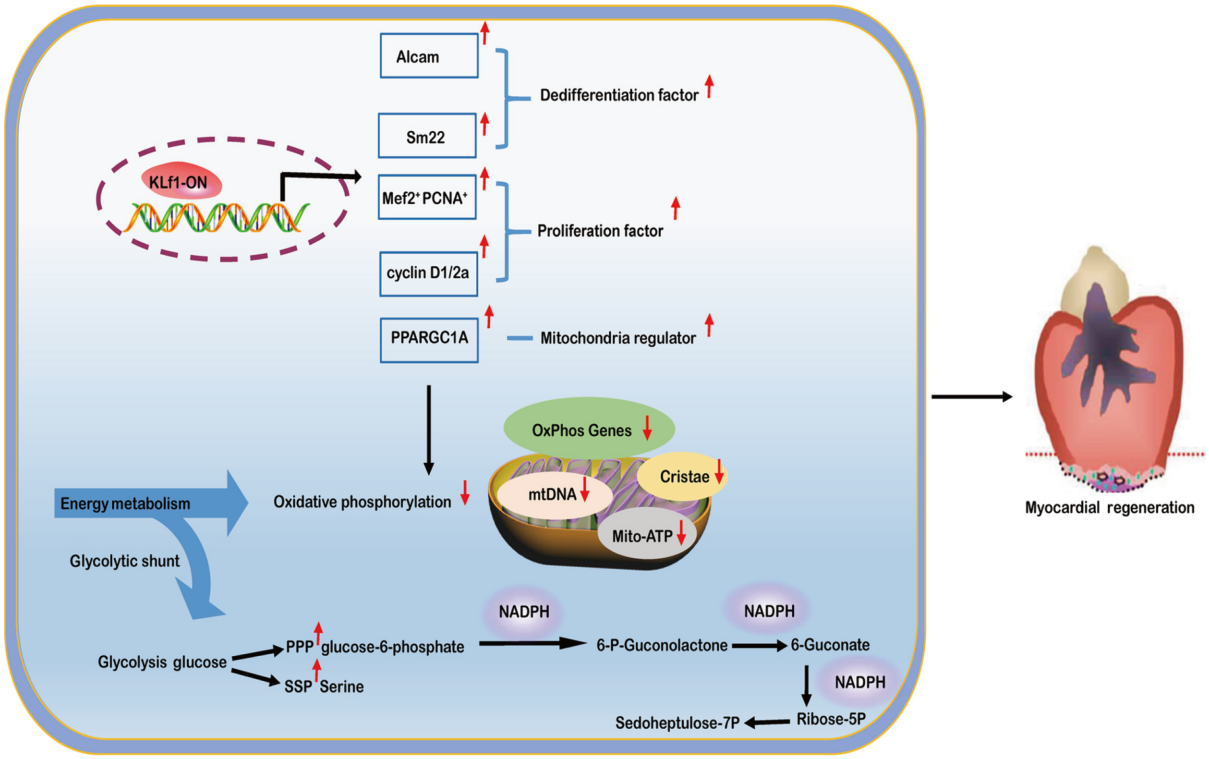

Figure 1. KLF1 triggers metabolic reprogramming to induce cardiomyocyte proliferation and myocardial regeneration KLF1, as a transcription factor, regulates the proliferation, differentiation and energy metabolism reprogramming of cardiomyocytes by regulating different factors. KLF1 can upregulate myocyte enhancer factor 2-positive proliferating cell nuclear antigen-positive ($Mef2^+PCNA^+$, cyclin D1/2a) and dedifferentiation factors (Alcam and Sm22) to promote cardiomyocyte proliferation. In addition, KLF1 downregulates the PGC1α/PPARGC1A gene to impair mitochondrial function and further triggers glycolytic shunt, so as to provide energy for cardiomyocyte proliferation and myocardial regeneration. $Mef2^+PCNA^+$: myocyte enhancer factor 2-positive proliferating cell nuclear antigen-positive; Sm22: smooth muscle protein 22a; PPP: pentose phosphate pathway; SSP: serine synthesis pathway.

reprogramming of cardiac transcription factor network. Myeloblastosis (MYB) is a family of transcription factors. C-myb is a key regulator of human primary hematopoietic stem/progenitor cell lineage. C-myb is highly expressed in immature and proliferating cells of all hematopoietic lines butdownregulated in the process of final differentiation. C-myb silencing can enhance the expression of KLF1, and c-myb–driven transactivation of KLF1 expression enhances erythropoiesis and regulates the fate of erythroid versus [10]. Meanwhile, Li *et al.* [11] believe that the c-myb protein encoded by MYB is involved in reactive oxygen species (ROS)-mediated cardiomyocyte injury. Therefore, we can infer that c-myb silencing can enhance the myocardial regeneration of KLF1 to a certain extent, which requires further evidence to confirm the relationship between c-myb and KLF1 in myocardial regeneration.

In conclusion, Ogawa *et al.* [1] discovered a novel function of KLF1 in myocardial regeneration and explored the metabolic reprogramming mechanism of KLF1 in the process of myocardial regeneration (Figure 1). Myocardial regeneration requires dedifferentiation and proliferation of mature cardiomyocytes, but the mechanism of energy metabolism reprogramming triggered by KLF1 is not completely clear. Understanding the process and mechanism of myocardial regeneration is crucial to biomedical research and offers the possibility of heart regeneration therapy. KLF1-dependent myocardial regeneration will provide a new reference for the study of cardiogenesis in hearts and may provide a therapeutic strategy for the better renovation of cardiac regeneration, which has a broad application prospect.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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