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Review

Cardiomyocyte proliferation, a target for cardiac regeneration☆

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A B S T R A C T

Cardiac diseases, characterized by cardiomyocyte loss, lead to dramatic impairment of cardiac function and ultimately to congestive heart failure. Despite significant advances, conventional treatments do not correct the defects in cardiac muscle cell numbers and the prognosis of congestive heart failure remains poor. The existence, in adult mammalian heart, of low but detectable cardiomyocyte proliferative capacities has shifted the target of regenerative therapy toward new therapeutical strategy. Indeed, the stimulation of terminally differentiated cardiomyocyte proliferation represents the main therapeutic approach for heart regeneration. Increasing evidence demonstrating that the loss of mammalian cardiomyocyte renewal potential shortly after birth causes the loss of regenerative capacities, strongly support the hypothesis that a detailed understanding of the molecular mechanisms controlling fetal and postnatal cardiomyocyte proliferation is essential to identify targets for cardiac regeneration. Here, we will review major developmental mechanisms regulating fetal cardiomyocyte proliferation and will describe the impact of the developmental switch, operating at birth and driving postnatal heart maturation, on the regulation of adult cardiomyocyte proliferation, all these mechanisms representing potential targets for cardiac repair and regeneration.

1. Introduction

Cardiovascular diseases are the leading cause of mortality in industrialized countries. Despite significant advances conventional treatments do not replace cardiomyocytes that are lost during the pathology and the prognosis of heart failure remains poor. For this reason, the replacement of dead cardiomyocytes is becoming the primary target for regenerative medicine research. The recent discoveries of adult cardiomyocyte proliferative capacities have orientated cardiovascular research toward therapeutical strategies that aim to activate adult cardiomyocyte cell cycle reentry. Although recent studies have uncovered the regenerative capacity of the newborn mammalian heart, this regenerative potential is lost shortly after birth, strongly supporting the hypothesis that a detailed understanding of developmental mechanisms is essential to identify targets for cardiac repair and regeneration.

While certain fishes or amphibians undergo complete adult heart regeneration following injury [1,2], the adult mammalian heart develops an extensive myocardial scar [3,4]. Nevertheless, mammalian newborn heart regenerative capacities have been recently uncovered. Indeed, Porrello et al. demonstrated that 1-day-old postnatal mice are able to regenerate their myocardium after apex resection and lineage tracing analysis revealed that neonatal mouse heart regeneration occurs through partial dedifferentiation and completed cell division of existing cardiomyocytes. This study nevertheless revealed that mouse heart postnatal regenerative capacities are lost within the first week of postnatal life [5]. Interestingly, the loss of mouse postnatal heart regenerative capacities coincides with decreased cardiomyocyte proliferation as cardiac growth transitions from hyperplasic to hypertrophic growth [6]. In addition to postnatal mouse heart regeneration, multiple evidence of postnatal human heart recovery post-injury have been reported. In 1937, postmortem histological analysis of a 6 years old child heart displaying myocardial dilation with necrotic area revealed mitotic cardiomyocytes at the bordure of the injured area suggesting the existence of potential human myocardial regeneration [7]. More recently, a case of a newborn child with massive myocardial infarction and subsequent severe cardiac damages has been reported to undergo complete functional cardiac recovery following myocardial reperfusion [8]. Postnatal regenerative potential has been also evaluated in pigs. Indeed, Zhu et al. demonstrated that the neonatal porcine heart is also able to regenerate. Notably the authors demonstrated that pig heart regeneration occurs when cardiomyocyte proliferation levels are high and is lost when cardiomyocytes exit the cell cycle at postnatal day 2 [9]. Thus, all these evidence strongly suggest that understanding the developmental mechanisms controlling cardiomyocyte proliferation will indisputably lead to the identification of novel targets for promoting adult cardiomyocyte proliferation and ultimately heart regeneration.

Heart development is a complex process that can be divided in two main phases of growth. The first phase consists in the addition of

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cardiac progenitors into the heart tube. These cardiac progenitors, located outside of the linear embryonic heart tube, are highly proliferative and differentiate as they enter the heart tube in order to elongate that one. Perturbations in transcriptional regulation and signaling pathways controlling cardiac progenitor deployment will lead to congenital heart defects [10,11]. The second phase of cardiac growth occurs at fetal stages. Combined with a remodeling process including septation of cardiac cavities and outflow track, fetal heart growth is exclusively achieved through the extensive proliferation of differentiated cardiomyocytes leading to the exponential increase of the cardiac volumes [12]. Correct morphogenesis of the heart requires a tight control of fetal cardiomyocyte proliferation, perturbations of which would lead to congenital heart defects [13].

At fetal stages, cardiomyocyte proliferation is maximal; and at that stages, cardiomyocytes undergo complete cytokinesis resulting in the hyperplasic growth of the heart. After birth, a transition from hyperplasic to hypertrophic growth is operating, and cardiomyocytes undergo mitosis without cytokinesis resulting in cell binucleation [14,15]. While mouse cardiomyocytes exit the cell cycle approximately one week after birth [16], the postnatal phase of hyperplasic growth seems to last for several years in the human heart. Indeed, cardiomyocyte cytokinesis activity has been detected up to 20 years of age [17].

In contrast to postnatal heart, adult mammalian heart has been, for decades, considered as post-mitotic. However, several studies have demonstrated that adult cardiomyocytes are able to replicate their DNA [18]. More recently, Bergmann et al., taking advantage of the integration of ^{14}C generated by bomb testing during the Cold War, revealed the evidence of low but detectable level of cardiomyocyte renewal in human adult heart with an estimated turnover from 1% to 0.3% per year [19]. In addition, ^{15}N isotope imaging approach, combined with genetic-lineage tracing study, clearly demonstrated that in mice, cardiomyocyte renewal during normal aging is achieved through complete cell division of pre-existing cardiomyocytes [20]. Altogether, these studies strongly demonstrate cardiomyocyte renewal throughout life and support the hypothesis that cardiomyocyte cell cycle reentry and division could be enhanced in the adult injured heart to promote cardiac regeneration. Nevertheless, this strategy requires the understanding of, on one hand, the molecular mechanisms promoting fetal and postnatal cardiomyocyte cell division, and on the other hand, those resulting in postnatal cardiomyocyte cell cycle exit.

Birth is characterized by multiple physiological and morphological changes that contribute to intense cellular remodeling including increased oxygen consumption associated with metabolic changes [21], modifications in the extracellular matrix composition [22] and profound transcriptional remodeling [6], that are known now to deeply contribute to the decrease in cardiomyocyte proliferation after birth. Here we will review the molecular mechanisms controlling cardiomyocyte proliferation at fetal stages and will describe the impact of the developmental switch driving postnatal heart maturation on the regulation of adult cardiomyocyte proliferation, all these mechanisms representing potential targets for promoting adult heart regeneration. We will particularly focus on the role of cell cycle components, the Hippo pathway, O_2 exposure associated with metabolism changes, extracellular matrix (ECM) and growth factors in the control of cardiomyocyte proliferation.

2. Cell cycle component regulation

The cell cycle can be divided into four different phases (G1-G2-S-M) that are specifically and tightly regulated by cyclins, cyclin-dependent kinases (CDK) and cyclin-dependent kinase inhibitors (CDKi). After birth, cardiomyocytes stop proliferating and exit from the cell cycle. The evaluation of cell cycle regulator expression patterns at fetal and postnatal stages led to a better understanding of cardiomyocyte cell cycle exit after birth [6] (Fig. 1). Ikenishi et al. demonstrated that cyclin and CDK expression, together with their complex formation and activation, evolve in a synchronous manner at fetal and postnatal stages. While elevated expression levels of cyclin E, A, B1 and D1 and CDK1, 2 and 4 and complex formation and activation are observed at fetal stages, they gradually decrease to reach a minimum level at postnatal day (P) 0 [23]. The decreased cyclin and CDK expression and the enhanced inactivation of the cyclin-CDK complex observed after birth are accompanied by increased expression of CDKi including $p21^{cip1}$ and $p27^{kip1}$ strongly participating to cardiomyocyte cell cycle exit [24,25]. FOXO transcription factors are known to regulate the expression of the Cip/Kip family of CDKi. *Foxo1* and *Foxo3* are expressed in the developing heart and at neonatal stages. In the adult heart, while Evans-Anderson et al. described low *Foxo1* mRNA levels and did not detect *Foxo3* mRNA [26], substantial levels of myocardial FOXO3 protein have been reported [27]. In a non-phosphorylated form, FOXO transcription factors translocate to the nuclei where they transcriptionally activate CDKi expression, leading to decreased cardiomyocyte proliferation. The IGF1/PI3K/AKT and FGF10 signaling pathways, by mediating FOXO1 and FOXO3 phosphorylation, respectively, promote embryonic cardiomyocyte proliferation [26,28]. Phosphorylated FOXO1 levels gradually decline after E18.5, coinciding with the increase in $p27^{Kip1}$ and $p21^{Cip1}$ expression [25,26]. Conditional overexpression of a constitutive active form of FOXO1 in fetal heart results in a thin myocardium, reduced cardiomyocyte proliferation and early fetal lethality. In contrast, conditional overexpression of a dominant negative form of FOXO1 activity in fetal cardiomyocytes leads to aberrant cardiomyocyte proliferation consistent with decreased CDKi expression [26]. The transcription factor MEIS1 through the activation of CDKi expression including *p15*, *p16* and *p21* has been shown to participate to postnatal cardiomyocyte cell cycle exit. Indeed, MEIS1 which expression increases at P1, switches localization from a perinuclear region at P1 to a nuclear localization at P7. *Meis1* deletion in cardiomyocytes is sufficient to extend the postnatal proliferative window and promotes cardiomyocyte mitosis in the adult heart [29]. All these studies suggest that the modulation of cell cycle regulator expression at adult stage may promote cardiomyocyte proliferation and may be beneficial in pathological conditions.

Conditional overexpression of cyclin D1 in the myocardium promotes cardiomyocyte DNA synthesis and multinucleation in adult heart [30] and the overexpression of the specific cyclin D2 is sufficient to promote cardiomyocyte proliferation and improved heart function following myocardial infarction [31,32]. More recently, by increasing the level of cyclin B-CDK1 and cyclin D-CDK4 complexes, Mohamed et al. demonstrated that P7 as well as adult cardiomyocytes exhibit enhanced proliferative rate with complete cytokinesis. Finally, intra-myocardial injection of this cyclin-CDK cocktail post-myocardial infarction leads to increased cardiomyocyte proliferation and reduced scar formation associated with improved cardiac function [33]. All these evidence thus demonstrate that modulating cell cycle components is thus a promising strategy to promote cardiomyocyte cell-cycle reentry in the injured heart.

3. Hippo pathway

The Hippo pathway is a conserved mechanism that restrains cell proliferation in order to control organ size. The Hippo pathway consists in a series of kinase cascade activation leading to the phosphorylation and inactivation of two transcriptional coactivators, named YAP and TAZ. When activated, YAP and TAZ bind to specific transcription factors including TEAD, resulting in the positive transcriptional regulation of genes implicated in cell cycle progression [34]. The Hippo signaling pathway also controls mammalian heart size. Indeed, the inhibition of the Hippo component Salvador, during heart development, leads to an increased cardiomyocyte proliferation resulting in cardiomegaly [35]. YAP expression is robust during development, in neonatal and juvenile heart but declines with age and is almost undetectable after 12 weeks of age [36] strongly correlating with its essential role in promoting fetal

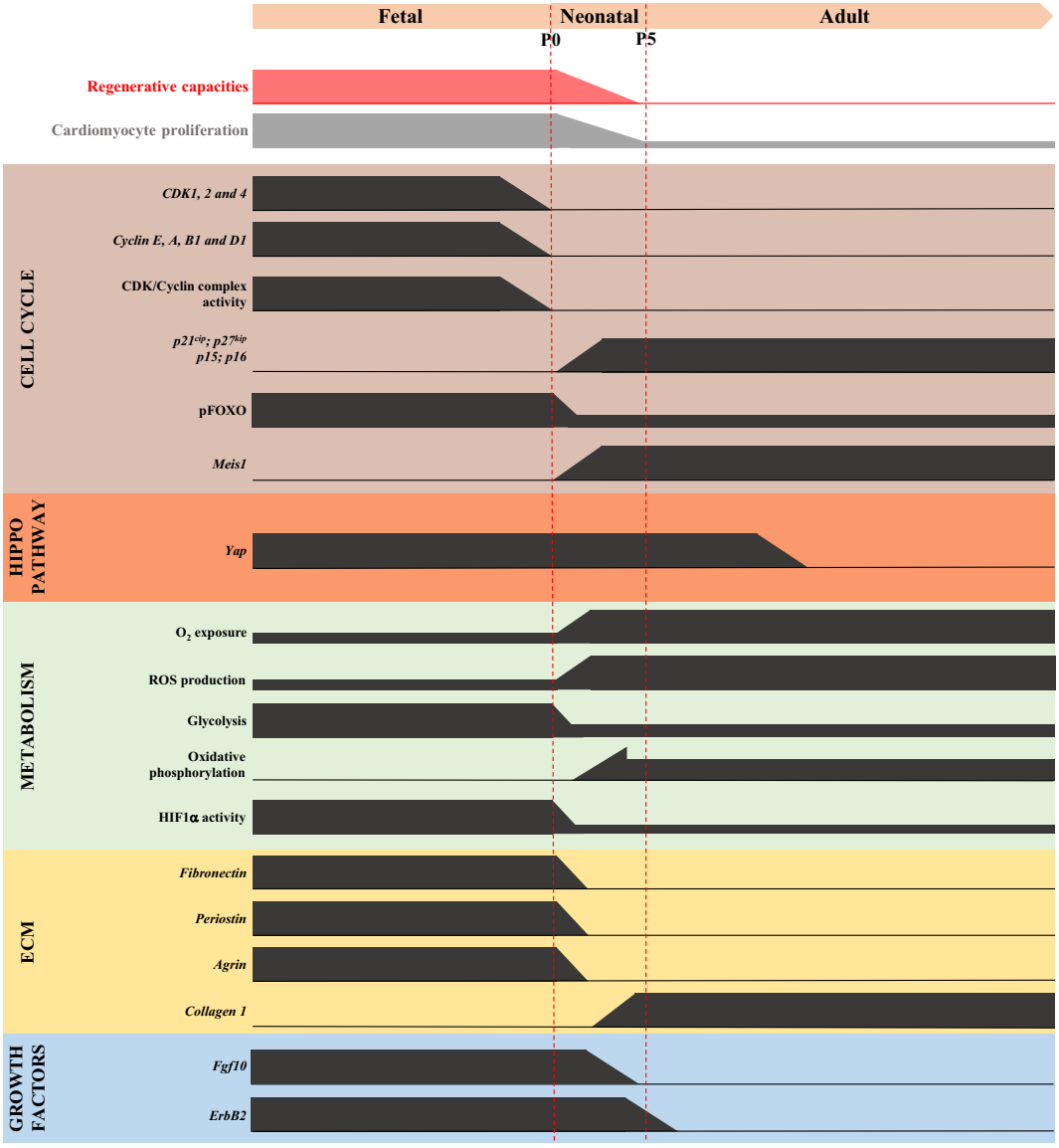

Fig. 1. Expression patterns of cardiomyocyte proliferation regulators in the fetal, neonatal and adult heart.

cardiomyocyte proliferation [37]. Indeed, cardiomyocyte-specific *Yap* loss of function mouse model substantially reduces fetal cardiomyocyte proliferation, leading to lethal cardiac hypoplasia at E16.5 [36]. FAT4, a protocadherin member, has been shown to modulate, through a non-canonical manner, the Hippo pathway to restrict heart growth. Indeed, by sequestering the Yap/AMOTL1 complex out of the nucleus, FAT4 prevents cardiomyocyte proliferation [38].

Small noncoding RNAs (miRNAs) regulate gene expression at the post-transcriptional level by degrading their mRNA targets. To date, multiple miRNAs have been identified to control both fetal cardiomyocyte proliferation and postnatal cardiomyocyte cell cycle reentry [39,40], partly through repression of Hippo core pathway components [41]. *In vitro* and *in vivo* experiments demonstrate that, by upregulating cell cycle gene expression including cyclin A2, cyclin B1 and CDK1, YAP gain of function is able to promote adult cardiomyocyte cell cycle reentry [36]. In addition, the modulation of Hippo pathway components including Salvador and YAP is sufficient to enhance postnatal and adult heart regeneration [40–45]. These evidence thus strongly support the Hippo pathway as a potential target to promote heart regeneration and repair (Fig. 2).

4. Oxygen exposure and metabolism

At birth, mammalian heart transitions from a low oxygen environment to an oxygen rich environment leading to a dramatic metabolic switch [21]. Indeed, the setting-up of breathing results in the lung bypass closure (foramen oval and the ductus arteriosus) causing a rapid raise in O_2 pressure in the aorta and an increased cardiac workload. To support these hemodynamic changes, profound cellular remodeling including maturation and hypertrophy of cardiomyocytes is essential [46]. These postnatal cellular changes require high energy production which will be provided by major rearrangements of energy metabolism switching from anaerobic glycolysis to oxygen-dependent mitochondrial oxidative phosphorylation [47] (Fig. 1). Glycolytic metabolism has been recently suggested to contribute to the hyperplasic cardiac growth. Indeed, glucose prevents human embryonic stem cell-derived cardiomyocyte maturation and promotes their proliferation. Interestingly, a drastic glucose uptake decline is observed at late gestation and after birth leading to intracellular glucose deprivation [48]. The postnatal metabolic switch increases ROS production, mitochondrial DNA content and DNA damage response leading to cardiomyocyte cell cycle arrest and maturation. Recently, Mills et al., using a cardiac organoid

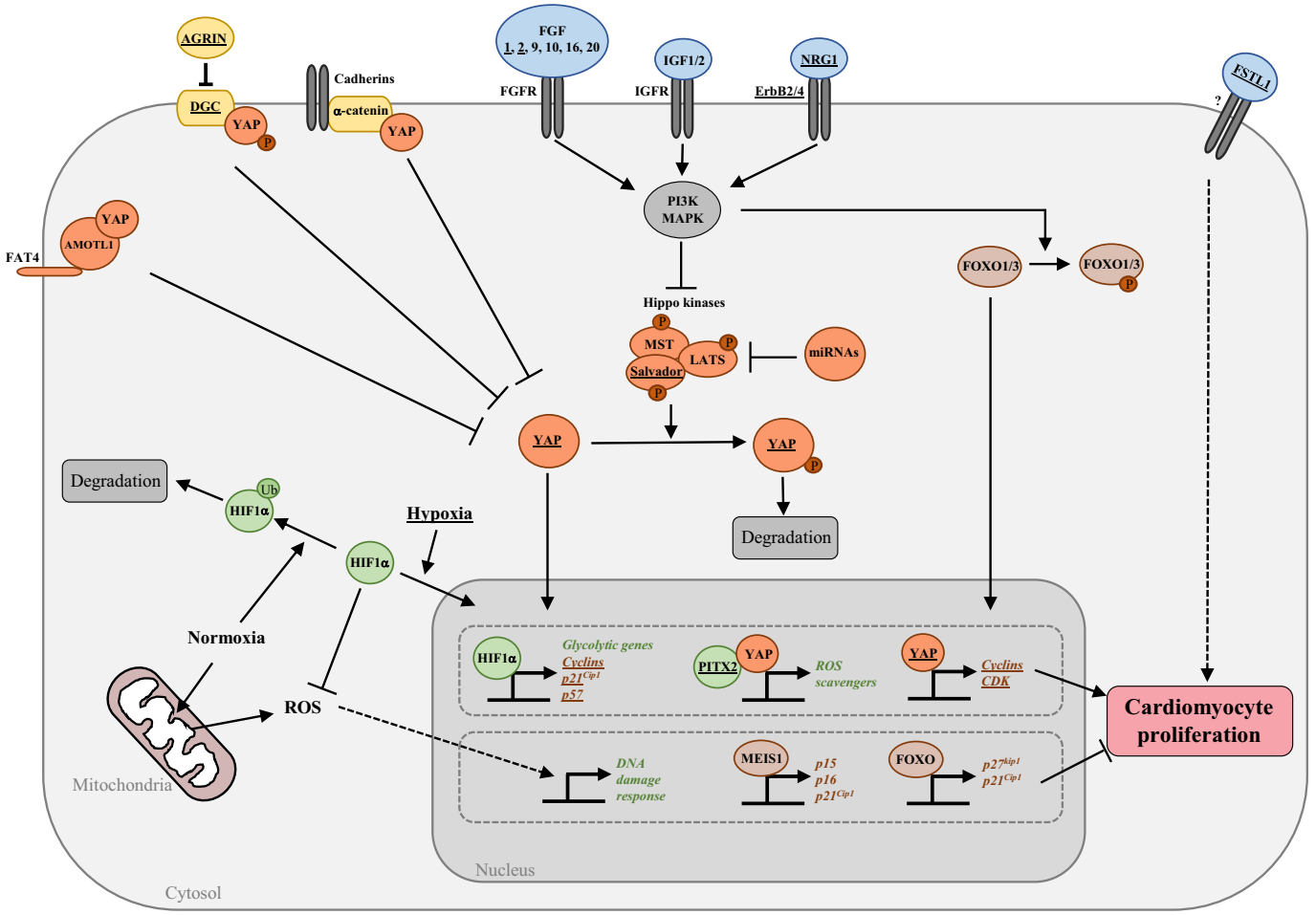

Fig. 2. Signaling pathways regulating cardiomyocyte proliferation. Each pathway involved in the regulation cardiomyocyte proliferation is highlighted: green for oxygen exposure and metabolic pathway, yellow for ECM and cell adhesion, brown for cell cycle components, blue for growth factor signaling and orange for Hippo pathway. Underlined elements represent pathways involved in cardiac regeneration. CDK, cyclin-dependent kinase; CDKi, cyclin-dependent kinase inhibitor; DGC, dystrophin–glycoprotein complex; ROS, reactive oxygen species. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

culture system, showed that switching metabolism from carbohydrates to fatty acids is a critical driver of cardiac maturation. Surprisingly, this proliferative barrier imposed by fatty acid metabolism could be removed by simultaneous activation of both β-catenin and YAP1 [49].

Interestingly, the maintenance of hypoxic conditions after birth leads to the prolongation of the cardiomyocyte proliferation postnatal window [50]. The Hypoxia Induced Factor 1α (Hif1α), the master transcriptional regulator of the adaptive response to hypoxia, has been strongly suggested to contribute to the regulation of fetal cardiomyocyte proliferation. At fetal stage, the low oxygen environment results in high Hif1α activity [51], and in addition to regulate gene expression involved in cardiomyocyte glycolytic status, Hif1α, has been shown to directly repress genes encoding cell cycle inhibitor including *p21* and *p57* and thus promotes proliferation [52]. Consequently, *Hif1α* mutant embryos display underdeveloped heart with myocardial hypoplasia due to a significant decrease in fetal cardiomyocyte proliferation [52,53]. At birth, mammalian heart transitions from a fetal hypoxic environment to a postnatal oxygen rich environment, leading to the inhibition of Hif1α activity [54]. However, in the adult heart, a rare population of hypoxic cardiomyocytes (Hif1α-responsive) remains. These hypoxic cardiomyocytes show typical features of proliferative cardiomyocytes including low oxidative DNA damage, smaller cell size, and mononucleation [55]. By displaying decreased *Meis* family member gene expression and upregulated levels of CDK/cyclins along with the downregulated expression of negative cell cycle regulators, the hypoxic cardiomyocytes predominantly contribute to cardiomyocyte renewal throughout life [55].

Patient and animal models with myocardial infarction, due to reduced oxygen availability in the ischemic region, present fetal metabolic gene re-expression causing the reversion of the energy metabolism from an oxygen-dependent mitochondrial oxidative phosphorylation to an anaerobic glycolysis [56–58]. This metabolic switch occurs extremely rapidly following myocardial infarction [59] and coincides with endogenous upregulation of cardiomyocyte proliferation [60]. Interestingly, the enhanced cardiomyocyte proliferation following myocardial infarction partly results from the significant increase in the number of cycling hypoxic cardiomyocytes [55]. Nevertheless, this endogenous response to hypoxia is not sufficient to face severe myocardial damages caused by the pathology [59]. All these evidence thus suggest that the modulation of the oxygen environment and subsequently metabolism may trigger cardiomyocyte cell-cycle reentry resulting in improved cardiac function and remodeling.

In 2002, Liao et al. showed that increasing myocardial glucose uptake protects against the progression to heart failure and improves survival in mice with chronic pressure overload [61]. Nakada et al. demonstrated that hypoxia exposure one week after the induction of myocardial infarction leads to an improved heart function associated with an increased cardiomyocyte proliferation and a decreased fibrosis deposition, highlighting a potential therapeutic role of hypoxia for heart regeneration [62]. The transcription factor *Pitx2* is expressed in

the developing embryo soon after gastrulation [63] and is crucial for regulating cardiac left-right asymmetry during development [64]. By limiting ROS production after myocardial infarction, *Pitx2*-gain-of-function in adult cardiomyocytes has been shown to promote cardiac regeneration. In this study, *Pitx2*-induced cardiac regeneration requires physical PITX2 interaction with YAP in order to cooperatively activate ROS scavengers [65]. In addition, PITX2 has been recently reported to be required to maintain, during heart regeneration, proper mitochondrial structure and function [66]. Overall these studies highlight the importance of environmental O_2 contents and subsequently metabolism status in the regulation of cardiomyocyte proliferation and ultimately heart regeneration (Fig. 2).

5. Extracellular matrix and cell adhesion

Adult cardiomyocytes are highly differentiated cells. Characterized by a rigid myoskeleton formed by organized sarcomeric structures, cardiomyocytes are tightly connected with each other through desmosomes, adherens, tight and gap junctions [67]. Thus, the extremely stiffened adult cardiomyocyte, to proceed complete cell cycle, has been shown to require partial dedifferentiation characterized by the disorganization of sarcomere structure and cell adhesion [39,68–70].

Cardiac fibroblasts which primary function is to produce structural proteins that comprise the extracellular matrix (ECM) [71], seem to play important role in the regulation of cardiomyocyte proliferation. Indeed, the composition and stiffness of the ECM surrounding cells is known to influence cardiomyocyte structure, fate and proliferative capacities [22,72]. While fetal and early neonatal heart ECM, enriched in fibronectin and periostin, is pro-proliferative and favors cardiomyocyte dedifferentiation [73,74], adult heart ECM, enriched in collagen 1, leads to more organized and non-proliferative cardiomyocytes (Fig. 1) [22]. In addition, while neonatal cardiomyocytes cultured on rigid matrix display increased nuclear division without cytokinesis and increased differentiation levels, neonatal cardiomyocytes cultured on soft matrix display dedifferentiation features including disorganized sarcomere network, smaller and rounding cardiomyocytes and cell cycle reentry [75]; strongly suggesting that extracellular matrix (ECM) remodeling may play a crucial role in the regulation of cardiomyocyte cell cycle progression. Recently, Bassat et al. discovered that ECM composition from P1 and P7 hearts have distinct properties regarding cardiomyocyte proliferation. Indeed, they demonstrated that P1 but not P7 ECM fragments lead to an increased cardiomyocyte proliferation suggesting that, rapidly after birth, modifications in ECM composition may participate to the loss of cardiomyocyte proliferation and subsequently loss of regenerative capacities [76]. In particular, they identified Agrin as a major ECM component. Indeed, Agrin through the disassembly of the dystrophin–glycoprotein complex (DGC) that sequesters YAP into the cytoplasmic compartment induces the activation of a YAP-related signaling mechanism which ultimately promotes cardiomyocyte partial dedifferentiation and proliferation [76,77] (Fig. 2). In addition to the modulation of the ECM composition, multiple lines of evidence suggest that the fibroblast-cardiomyocyte dialog is essential for the regulation of fetal cardiomyocyte proliferation. Indeed, though paracrine mechanisms, cardiac fibroblasts promote fetal cardiomyocyte proliferation and thus participate to fetal heart growth [73,78]. The adherens junction protein, α-catenin, has been also shown to play a role in cardiomyocyte maturation and proliferation. In fact, *α-catenin* deletion leads to an increased cardiomyocyte proliferation through the nuclear translocation of YAP both in perinatal and adult hearts [79] (Fig. 2).

Heart diseases are associated with a massive ECM remodeling resulting in an extensive non-conductive scar formation increasing matrix rigidity and reducing electrical property that ultimately impairs cardiac function [80]. Unlike mammals, lower vertebrates including zebrafish are able to proceed complete heart regeneration after injury. Nevertheless, in contrast to mammalian heart, the massive fibrotic scar developed in the zebrafish heart after damage is progressively resolved prior to complete myocardial regeneration [81]. Recent evidence suggest that in the injured zebrafish heart, while fibrotic scar elimination is required for complete heart regeneration, fibroblast-derived signals are essential for cardiomyocyte proliferation. Indeed, the ablation of ECM-producing cells in the injured zebrafish heart dramatically impairs cardiomyocyte proliferation [82]. Modifications in the ECM composition of the mouse infarcted heart, through the single intramyocardial injection of Agrin, were sufficient to improve cardiac function and promote heart regeneration [76]. However, while dystrophin loss of function mouse model failed to regenerate following apex resection of the neonatal heart, compound mutants with Salvador loss of function (Hippo pathway component) led to efficient cardiac repair [77] strongly suggesting that Agrin may not only act through the disassembly of DGC complex but may also directly prevent YAP phosphorylation. Thus, all these evidence strongly suggest that in pathological conditions, the modulation of the ECM composition, leading to a more compliant cardiomyocyte microenvironment, may be essential to promote cardiomyocyte cell cycle reentry and heart regeneration.

6. Growth factors

Due to their direct action on multiple cellular functions including cell adhesion, proliferation, differentiation and migration, the role of growth factors in the regulation of cardiomyocyte proliferation has been extensively studied. Diverse fibroblast growth factors (FGF) have been implicated in the regulation of fetal and neonatal cardiomyocyte proliferation. Indeed, *in vitro* experiments showed that FGF1, in combination with p38 MAP kinase inhibition [83,84], or FGF2 treatment [85], promote postnatal cardiomyocyte proliferation. FGF9, FGF16, and FGF20 signals originating from the endocardium and the epicardium have been shown to regulate fetal cardiomyocyte proliferation [86–88]. FGF10, which expression gradually declines after birth (Fig. 1) and coincides with postnatal decreased cardiomyocyte proliferation, has been reported to specifically regulate, through the phosphorylation of FOXO3 and the inhibition of $p27^{Kip1}$ expression, fetal cardiomyocyte proliferation [28]. Insulin-like growth factors (IGF) which are expressed by embryonic epicardial cells also play a role in cardiomyocyte proliferation [89]. *In vitro* and *in vivo* experiments demonstrate that both IGF1 and IGF2, through the activation of the PI3K/AKT downstream signaling cascade, stimulate the proliferation of fetal cardiomyocytes [26,90,91]. The Neuregulin 1 (NRG1) growth factor and its specific tyrosine kinase receptors, ErbB2/4, have been described to promote, through the activation of the PI3K downstream signaling, postnatal mononucleated cardiomyocyte proliferation [68,92]. Interestingly, potential role of the PI3K signaling pathway in the activation of YAP activity [93,94] may suggest that growth factor control of cardiomyocyte cell cycle progression might involve the Hippo block removal (Fig. 2).

The NRG1/ErbB2/4 signaling induces adult cardiomyocyte cell-cycle reentry [92] and the constitutively active form of the NRG1 receptor ErbB2, due to extensive cardiomyocyte proliferation, leads to cardiomegaly in adult heart [68]. As described above, the epicardial layer, by providing growth factors as mitogens contributes to myocardial growth during development. *In vitro* experiments demonstrated that adult epicardial-derived cell conditioned media increases the proliferation of adult cardiomyocytes and identified the Follistatin-like-1 (FSTL1) protein as an epicardial mitogenic factor [95]. Finally, FGF10 overexpression in adult heart leads to an increased cardiomyocyte proliferation [28]. All these lines of evidence demonstrating that growth factors not only enhance fetal cardiomyocyte proliferation but also promote adult cardiomyocyte cell cycle reentry, strongly suggest that growth factors may represent putative targets to promote cardiac regeneration in the injured heart.

After myocardial infarction, FGF1/p38 inhibitor injection enhances proliferation of both cardiomyocytes and endothelial cells resulting in improved heart function [83]. FGF2 overexpressing mice display enhanced fibroblast and endothelial cell proliferation and cardiomyocyte

hypertrophy leading to a preserve ejection fraction following myocardial infarction [85]. The administration of IGF1 after ischemia/reperfusion decreases cardiomyocyte cell death, prevents cardiac fibrosis and preserves cardiac function [96,97]. The application, in myocardial infarcted heart, of FSTL1-loaded patches mimicking epicardial reconstitution leads to an improved cardiac function with a decreased fibrosis, increased vascularization and cardiomyocyte regeneration [95]. Finally, NRG1 administration or constitutive activation of the NRG1 receptor ErbB2 following myocardial infarction leads to an improved cardiac function associated with decreased infarct size and increased cardiomyocyte proliferation [68,92]. All these lines of evidence demonstrating the crucial role of growth factors in regulating both fetal and adult cardiomyocyte proliferation and their beneficial effect after injury on heart function further support their use for cardiac regenerative therapeutical strategies.

7. Conclusions

Uncovering the molecular mechanisms controlling cardiomyocyte proliferation in the fetal heart and identifying the impact of cardiac morphological and physiological changes that operate at birth on those molecular determinants have not only enable to better understand cardiomyocyte cell cycle exit but clearly led to unveil potential targets for cardiac regeneration and repair. Heart diseases not only result in severe loss of cardiomyocytes but also display profound remodeling, including dramatic fibrosis deposition. As reviewed here, multiple studies revealed that favoring cardiomyocyte renewal post-injury is sufficient to reduce scar formation. Whether the molecular mechanisms employed to promote cardiomyocyte cell cycle reentry directly influence cardiac fibroblast activation has not been elucidated. The emergence of adeno-associated virus (AAV) technologies [98] may be a valuable strategy to genetically promote cardiomyocyte proliferation in Human. To date gene therapy for heart failure by using AAV gene vectors are currently into clinical trials [99,100]. Epicardial patches, currently used to provide exogenous cardiac cells toward the injured site [101,102], may also represent an interesting option to administrate paracrine factors known to target key regulators of cardiomyocyte proliferation [103]. This review highlights the crucial role of diverse mechanisms including cell cycle regulators, Hippo pathway, oxygen exposure, energy metabolism, ECM and growth factors in the regulation of cardiomyocyte proliferation and cardiac regeneration. Interestingly, a majority of these mechanisms seems to converge on the Hippo signaling pathway (Fig. 2) strongly suggesting that the release of the Hippo block operating in mature cardiomyocytes is a crucial checkpoint to enable cardiomyocytes to reenter the cell cycle.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgments

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