

## Research Highlight

# The combination of four cell-cycle regulators: a new direction for inducing adult cardiomyocyte proliferation

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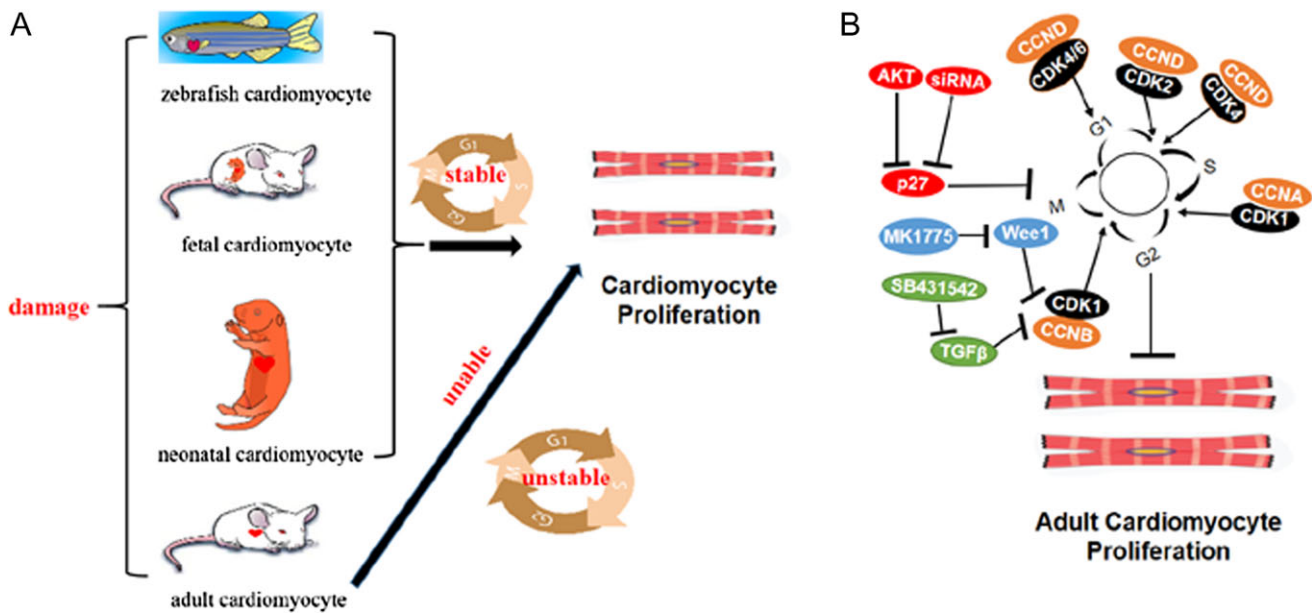
Cardiomyocytes, also known as myocardial fibers, are the muscle cells which form the heart tissue. Previous studies have indicated that fetal mammalian cardiomyocytes maintain the regeneration capacity, which promotes the fetal heart growth. Regardless of environment insults including nutrient deprivation, changes of blood flow, along with mechanical and volume loading [1], embryonic mammalian cardiac muscle cells are also related to robust proliferation response. Similarly, the hearts of 1-day-old neonatal mice could also be fully regenerated after surgical resection of the left ventricular apex or myocardial infarction (MI) [2]. Intriguingly, studies have also shown that certain fish, such as adult zebrafish, or urodele amphibians retain an observable capacity for regeneration [3]. In response to cardiac damage, zebrafish exhibits complete regeneration primarily due to the proliferation of cardiomyocytes. Nevertheless, the mouse heart loses this potential in the first week after birth. Tragically, it has been demonstrated that the adult mammalian cardiomyocyte unable to proliferate (Fig. 1A). Adult heart is considered as a terminally differentiated organ [4] that has limited capacity for cardiomyogenesis. Therefore, patients suffering from cardiovascular failure are unable to repair the heart and survive after MI or other heart diseases. Therefore, finding a feasible approach to stimulate adult mammalian cardiomyocyte proliferation is beneficial for the treatment of MI and other heart diseases.

As we all know, cell cycle is the complete cell process from the completion of one mitosis to the end of the next split, which is regulated by a series of cell-cycle regulators. Cyclins and their catalytic partners, the cyclin-dependent kinases (CDKs) play central roles in this process. At a specific stage of cell cycle, the heterodimeric cyclin-CDK complexes phosphorylate a set of cellular proteins and further promote these phosphorylated proteins to enter the progression through the G1 phase and drive DNA synthesis. Most importantly, cyclin-CDK complexes also trigger the segregation of the newly formed double-stranded chromosomes to the daughter cells in

mitosis, thereby ensuring the cell-cycle progress [5]. Nevertheless, it has been found that only a few cyclin-CDK complexes directly participate in the cell division cycle. For example, the combination of CDK2 and cyclin D (CCND) plays an important role in G1-S phase. CDK1-cyclin A (CCNA) complexes are involved in the S-G2 phase, while CDK1-cyclin B (CCNB) complexes are responsible for promoting the G2-M phase and inducing cardiomyocyte karyokinesis. CDK4-CCND complexes promote the G1-S phase and cardiomyocyte DNA synthesis. At the same time, CCND-CDK4 and CCND-CDK6 complexes phosphorylate and inactivate pocket proteins, thereby enabling the transcription of genes that participate in cell-cycle progression (Fig. 1B).

Although it has been well established that cyclin-CDK complexes drive cell-cycle progression, mammalian cyclins and CDKs may also play important roles in other cellular events [6], such as gene transcription, DNA damage repair, cell death, cell differentiation, immune response, and metabolism. A previous report also showed that overexpression of cyclins and CDKs are implicated in human tumors [7].

Recently, Mohamed *et al.* [6] reported that overexpression of CDK1, CDK4, CCNB, CCND, and a combination of cell-cycle regulators, efficiently induce adult cardiomyocyte proliferation and subsequent cell survival *in vitro* and *in vivo*. CDK1, CCNB, CDK4, and CCND play an important role in the post-mitotic cell proliferation. They further revealed that CDK1, CCNB, and aurora kinase B induce the most of the cardiac cells that undergo complete cell division [6]. By comprehensively testing the cell-cycle regulators, they observed that the combination of CDK1-CCNB complexes and CDK4-CCND complexes most efficiently contribute to the onset of the adult cardiomyocyte proliferation. Furthermore, they explored whether the combination of CDK1-CCNB-CDK4-CCND notably induce the adult cardiomyocyte division *in vivo* through lineage tracing method to track cellular proliferation, differentiation,



**Figure 1. The inhibition of Wee1, TGF $\beta$ , and p27 indirectly promote the proliferation of adult cardiomyocytes** (A) The fetal and neonatal mammalian cardiomyocytes possess a robust proliferation capacity after cardiac damage during early of life. Similarly, zebrafish cardiomyocytes harbor a robust regeneration capacity after cardiac damage. The capacity is primarily due to undergoing stable cell-cycle process. In contrast, adult cardiomyocytes have a limitation to proliferation after cardiac damage due to inability re-entry into the cell-cycle process. (B) Cell-cycle progression is promoted by the heterodimeric cyclin-CDK complexes. Wee1 inhibitor MK1775 and the transforming growth factor  $\beta$  (TGF $\beta$ ) inhibitor SB431542 are able to inhibit Wee1 and TGF $\beta$ , respectively, thereby indirectly promoting the progression of CDK1/CCNB involved in the cell cycle. Small interfering RNA (siRNA) knocks down p27 protein or activates the AKT and further down-regulates p27 protein, indirectly promoting the proliferation of adult cardiomyocytes.

migration, and all of its progeny. Their results ultimately revealed that 15%–20% of adult cardiomyocytes showed successful division, along with cardiac function obviously improved after myocardial infarction (MI). Of particular note, histological analyses also showed the same results. Moreover, overexpressions of CDK1-CCNB-CDK4-CCND complexes contribute to the degradation pathway. The primary reason is that the degradation of proteasome-dependent proteins limits the activities of the cell-cycle regulators, thereby resulting in declined protein products over time.

To explore the mechanism that promotes the proliferation of adult cardiomyocytes, MK1775 (Supplementary Fig. S1) is a chemical inhibitor of Wee1 and a negative regulator of CDK1. SB431542 (Supplementary Fig. S1) is an inhibitor of transforming growth factor  $\beta$  (TGF $\beta$ ). Both MK1775 and SB431542 are capable of inhibiting Wee1 and TGF $\beta$ , respectively, thereby promoting the progression of CDK1-CCNB involved in the cell cycle (Fig. 1B). Chemical inhibition of Wee1 and TGF $\beta$  makes CDK1-CCNB dispensable. They further revealed that the p27 protein participates in the progress from G1-S to G2-M phase. As we all know, the normal progression of the cell cycle is closely associated with the orderly expression and regulation of CDKs. Cyclin-dependent kinase inhibitor p27 protein (also known as KIP1) is a factor that inhibits the progression of cell-cycle. A previous study suggested that p27 plays a dominant role in cell proliferation, differentiation, apoptosis, epigenetic modifications, and post-translational modification [8]. Mohamed *et al.* [6]. inhibited the p27 protein expression by using small interfering RNA, which was found to indirectly promote the proliferation of adult cardiomyocytes (Fig. 1B). In addition, a recent research reported that the PI3K/AKT signal pathway also participates in p27 function. Activation the AKT leads to the phosphorylation of p27, resulting in the inhibition of cell-cycle progression in

cholangiocarcinoma [9]. Therefore, AKT could inhibit the overexpression of p27 and then promote the proliferation of adult cardiomyocytes (Fig. 1B).

Nevertheless, there are still a series of limitations in stimulating adult cardiomyocyte proliferation. Firstly, the combination of CDK1-CCNB-CDK4-CCND is too complicated, which may limit its application in clinic. For example, it is so difficult that control the number of CDK1-CCNB-CDK4-CCND involved in regulating the cell cycle, thereby resulting in the human system dysfunction and bringing greater hazards on patients with cardiac diseases. Secondly, the specificity of the CDK1-CCNB-CDK4-CCND complexes in regulating adult cardiomyocyte cell-cycle progression is still a problem. These cell-cycle regulators may also act on other cells, thereby causing overproliferation of other cells, which may lead to the development of tumors. Even with these limitations, inducing adult cardiomyocyte proliferation through cell-cycle regulation may still be a new idea, which provides a good foundation for future research.

## Supplementary data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

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