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Oxygen

Double-Edged Sword in Cardiac Function and Repair

Yen-Sin Ang, Deepak Srivastava

**The Oxygen-Rich Postnatal Environment Induces
Cardiomyocyte Cell-Cycle Arrest Through DNA
Damage Response.**

Puente et al

Cell. 2014;157:565–579

Mammalian hearts transition from a hypoxic in utero environment to an oxygen-rich habitat after birth, coinciding with the rapid postnatal exit of cardiomyocytes out of the cell cycle. A recent article in *Cell* identified a novel connection between the 2 seminal events, focusing on the accumulation of reactive oxygen species in cardiomyocytes as a major mediator of the critical neonatal cell cycle switch.

Among the 11 major organ systems of the human body, only a selected few are capable of regenerating after organ damage, with considerable disparity in efficiency between amphibians, fishes, and mammals. The skin, bone marrow, intestine, and liver regenerate relatively well, and a common gecko restores its tail more effectively than a fish can repair its tailfin.^{1,2} In humans, skeletal muscle has the ability to heal from injury. In contrast, cardiac muscle, which shares many common features, has limited capacity to regenerate after damage. What determinants account for these differences, and what can we learn from each organ or animal so we can attempt to recapitulate such factors in poorly reparative tissues?

Currently, there are ≥ 2 potential avenues by which the limited amount of cellular replenishment may occur in the mammalian heart. The first involves the proliferation and differentiation of resident cardiac progenitor cells bearing markers, such as cKit, Sca1, and Isl1. Self-renewing progenitor cells expressing some or all of these markers exist in defined microenvironments in vivo and possess properties of clonal expansion and multipotency ex vivo. Incongruent reports have suggested that, although clonal Lin⁺cKit⁺ cells isolated from adult mouse hearts can repopulate the myocardium after cardiac injury (myocardial infarction),^{3,4} the frequency at which

this phenomena occurs is extremely low and thus arguably insignificant.⁵ An alternative source for new cardiomyocytes in the postnatal mammalian heart may involve reactivation of terminally committed cardiomyocytes to re-enter the cell cycle. Multiple groups have identified rare cycling cardiomyocytes in normal and pathological postnatal hearts.^{6,7} However, the vast majority of cardiomyocytes irreversibly exit the cell cycle soon after birth, switching from a hyperplastic into a hypertrophic growth state.⁸ Although factors, such as Cyclin-A2 and Meis1, have been implicated,^{9,10} the environmental cues, the signaling pathways, and gene regulatory networks that regulate the switch between these cellular states are poorly understood.

In a recent article by Puente et al,¹¹ the authors made a surprising connection between the barrier to re-enter cell division and the oxygen-rich environment in which most mammals exist postnatally. They made an astute link between 2 observations: (1) zebrafish, which regenerate effectively after ventricular resection, live in a relatively hypoxic (PaO₂-15 mmHg) environment under water and (2) resected neonatal mouse hearts lose their ability to regenerate soon after being born into an oxygen-rich (PaO₂-100 mmHg) atmosphere compared with the more hypoxic in utero environment. The study built on the initial observation that there was a time-dependent increase in mitochondrial activity, reactive oxygen species (ROS) production, and the DNA damage response shortly after birth. Because ROS production is one indication of the extent of oxygen consumption, these results prompted the authors to inquire whether the increase in environmental oxygen content might directly augment the DNA damage response, which may then induce cardiomyocyte cell-cycle arrest. To test this, they challenged neonates to hyperoxic and hypoxic conditions and measured cell proliferation by measuring 2 markers: phosphorylated histone H3 and AuroraB. In agreement with their hypothesis, hyperoxia induced a dramatic reduction in phosphorylated histone H3 levels in cardiomyocytes, whereas hypoxia triggered the opposite effect. Interestingly, the degree of augmentation of AuroraB levels was significantly less pronounced, suggesting that low environmental oxygen induced cardiomyocytes to initiate the cell cycle but might be insufficient to complete cell division.

To test the effects of increased ROS and oxidative stress on cardiomyocyte proliferation, they further injected neonates with agents of ROS generation (diquat, paraquat, and H₂O₂), as well as scavengers of ROS (NAC), shortly after birth. Consistently, the authors reported accelerated cell-cycle arrest and marginal increases in cardiomyocyte size, indicating a fast-track switch into hypertrophic growth when ROS production was enhanced, and a delay in cell-cycle arrest when ROS was scavenged away. To rule out a cell nonautonomous and indirect response of cardiomyocytes to oxidative stress, an elegant genetic approach was used to scavenge for ROS specifically in cardiomyocytes. This was done by inducing a mouse-specific catalase, which catabolizes H₂O₂ into water and oxygen, using Cre recombinase under

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Commentaries serve as a forum in which experts highlight and discuss articles (published here and elsewhere) that the editors of *Circulation Research* feel are of particular significance to cardiovascular medicine.

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control of the cardiac-specific Myh6 promoter. Surprisingly, the percentage of proliferating cardiomyocytes was only a small subset within the cells that were overexpressing the catalase, signifying that clearance of ROS is one of several mechanisms regulating cell-cycle progression. Building on these data, the Sadek group performed the next critical and obvious experiment: they induced ischemia followed by reperfusion in relatively young mice and attempted to ameliorate the injury with concomitant ROS scavenging. Encouragingly, priming young mice with daily doses of NAC blunted the effects of the ischemia. Finally, the authors demonstrated that chemical inhibition of the G2/M checkpoint regulator, Wee1, was somewhat sufficient to delay cardiomyocyte cell-cycle arrest; however, how oxygen content and DNA damage response tie in with this result is unknown.

This study revealed the previously unrecognized significance of oxidative stress on postnatal cardiomyocyte cell-cycle exit. However, some interesting questions remain. First, the authors had largely used immunohistochemistry of Aurora B kinase as a proxy for completion of cytokinesis. However, because the broader Aurora B complex, including survivin and INCENP (inner centromere protein), localizes to centromeres and mitotic spindles throughout mitosis, it may not be the most bona fide marker of cytokinesis.¹² In light of the <20% increase in overall cell numbers on the treatment of NAC in vitro, better quantitative methods or time-lapse imaging of cardiomyocyte division would provide more conclusive evidence that cardiomyocytes are indeed proliferating after ROS scavenging. Second, in most experiments, the authors used an indirect measurement of ROS to indicate that the mouse hearts were truly experiencing hyperoxia or hypoxia. However, they did not provide direct evidence that the cardiac cells were indeed consuming more or less oxygen. For example, although the animals were incubated in a hypoxic atmosphere, compensatory responses may keep oxygen uptake and usage unchanged. Short-term in vitro culture of cardiomyocytes in hypoxia, followed by direct oxygen consumption measurements, might resolve this question.¹³ In addition to measuring oxygen consumption directly, it will be important to measure the extent of DNA damage directly although this may require single-cell approaches to reflect the presence of random DNA damage and potential consequences on the transcriptome accurately, including the interplay with hypoxia-inducible factor.¹⁴ Importantly, it remains to be determined whether the human cardiomyocyte cell-cycle is under similar mechanistic control.

In addition to providing novel insight into the mechanisms underlying the dramatic neonatal switch that occurs in the proliferative potential of cardiomyocytes, there are potential practical consequences of the observations made by Puente et al.¹¹ Because a metabolic switch from glycolysis to oxidative phosphorylation occurs postnatally, it is interesting to consider whether forcing cardiac cells to use glycolytic substrates or reducing their dependence on the Krebs cycle as an energetic pathway might maintain cardiomyocytes in a proliferative state.¹⁵ Recently, large numbers of human embryonic stem cell-derived cardiomyocytes were injected into injured macaque hearts, and this was necessary because of the significant loss of cells on injection.¹⁶ It is possible that the addition of ROS scavenging agents into existing stem cell differentiation cocktails may increase cardiomyocyte production by multiple folds by maintaining a more proliferative state.

The etymology of the Chinese character for to heal (愈) dates back 2300 years to the Warring States period. Encompassed in this character form is the word for heart (心), prophetically suggesting that the ancient Chinese healers recognized the inherent importance yet operose task in the repair and regeneration of this 1 human organ. Centuries later, this importance remains paramount, yet the operosity remains to be enlightened—we can only hope that studies such as this will point to new approaches to address this age-old problem.

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Disclosures

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