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EDITORIAL COMMENT

Hypoxia Induces Cardiomyocyte Proliferation in Humans*

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The mammalian heart has a substantial regenerative capacity in the first few days of life mediated by cardiomyocyte proliferation; however, this capacity is lost shortly after birth (1). Transition from the embryonic to postnatal circulation results in a rapid and substantial increase in oxygen tension coinciding with separation of the right and left sided circulations (2). This is accompanied by a shift from anaerobic glycolysis to mitochondrial oxidative phosphorylation, particularly toward fatty acid (FA) utilization by mitochondria. Previous studies have demonstrated that changes in oxygenation and metabolism are important regulators of cardiomyocyte maturation and cell cycle (3). For example, the postnatal window of cardiomyocyte proliferation in mice can be modulated by changes in the fraction of inspired oxygen immediately after birth, where hypoxia prolongs the window and hyperoxia shortens it (3). Similarly, exposure to gradual severe hypoxia results in reversion to anaerobic metabolism, decreased DNA damage, and reactivation of cardiomyocyte proliferation in adult mice (4). However, these studies and others were performed in rodents or in cultured cells, and it is unknown whether a similar phenomenon occurs in the human heart.

In this issue of *JACC: Basic to Translational Science*, Ye et al. (5) showed in an elegant report that hypoxia induces cardiomyocyte proliferation in the human heart in a population of patients with cyanotic heart diseases. The authors collected 30 ventricular outflow myocardial tissue specimens (obtained from the surgical procedure required to relieve obstruction) from Tetralogy of Fallot patients and categorized them into 3 main groups based on SaO_2 levels: mild hypoxia >85%, moderate hypoxia 75% to 85%, and severe hypoxia <75%. The authors are to be commended for the careful patient selection, which included parameters like age and sex matching as well as matching pulmonary pressure. They found that moderate hypoxia reduced mitochondrial oxidative DNA damage, reduced DNA damage response, and promoted cardiomyocyte proliferation. The authors also demonstrate a similar role of Hippo pathway in regulation of cardiomyocyte proliferation, not unlike previous landmark studies by Jim Martin's group (6) that implicated the antioxidant role of Pitx2 in regulation of cardiomyocyte proliferation. Overall, these results are well in line with previous observations in rodents. However, there is a significant unanswered question here: why are lower levels of oxygen associated with increased oxidative DNA damage? It is well accepted that acute hypoxia can result in bursts of mitochondria-derived reactive oxygen species, which might have been the case here. The current paper does not provide data with regard to fluctuations in oxygen levels in Group C patients, for example, which would at least partially explain these findings. Nevertheless, the mere demonstration of the correlation among hypoxia, decreased DNA damage, and human myocyte proliferation in vivo is a significant finding that moves the field forward.

The authors also performed studies on human induced pluripotent stem cell (iPSC)-derived cardiomyocytes. They exposed the cells to 1 of 4 levels of oxygen: 21%, 15%, 10%, or 5%. Oxygen levels were

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dropped every 2 days. They also showed that in the moderate range of hypoxia there is an increase in cardiomyocyte proliferation. Again, and similar to their in vivo results, the peculiar finding here was that lower levels of oxygen increase DNA damage. Although these results are also in line with hypoxia-induced regeneration studies in rodents, in our view these types of in vitro studies are difficult to interpret for several reasons. First, myocytes in culture are not exposed to mechanical stress, and it is well established that under regular culture conditions, these cells lack critical metabolic characteristics of adult cardiomyocytes. For example, a recent report by Porrello and Hudson's groups (7) showed convincingly that under regular culture conditions, levels of DNA damage in iPSC-derived myocytes are low, and that forcing fatty acid oxidation in the absence of changes in oxygenation is critical for induction of an oxidative metabolic phenotype and enhanced differentiation of iPSC-derived myocytes. Second, how does one translate an in vivo systemic oxygen level to that at a cellular level? For example, although 7% FiO_2 in mice for 2 weeks can induce myocyte proliferation in vivo, and in the current study by Ye et al. (5), 75% to 80% systemic arterial saturation was associated with myocyte proliferation, what is the equivalent oxygen level in cultured cells? The short answer is that we simply do not know. Third, previous studies have shown that it is not the oxygen level per se, but rather the metabolic programming that occurs in response to these oxygen levels that affects myocyte proliferation (3). Therefore, rapid changes in oxygenation over 2 days might not be sufficient to affect metabolic reprogramming. Finally, was there a differential effect on ROS production, whether mitochondrial or otherwise, at different oxygen levels? Thus, the level of hypoxia, the timeline of induction of hypoxia, the duration under hypoxia, and the mechanism of changes in DNA damage are all necessary factors to consider before reaching conclusions about a certain protocol of hypoxia in vitro. The authors thus suggest in their discussion that the protocol they used in vitro was perhaps too rapid to induce sufficient metabolic reprogramming, as an attempt to explain the discrepancy between their in vitro data and the in vivo data in mice. This might be true; however there simply is no way to correlate workload and oxygenation in a working heart in vivo to unloaded single-layer cardiomyocytes in vitro.

In conclusion, the findings presented in this report by Ye et al. (5) represent an important extension of the role in hypoxia in cardiomyocyte cell cycle regulation to the human heart, and they solidify the role of hypoxia in blunting DNA damage as a mechanism of myocyte proliferation. The studies also highlight the notion that careful consideration of the degree of hypoxia and the models used is warranted. Not all hypoxias are created equally.

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