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Lamin B2, Guardian of Cardiomyocyte Nuclear Division

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https://doi.org/10.1016/j.devcel.2020.03.016

Regenerative capacity is robust in the neonatal mouse heart but is lost during postnatal development when cardiomyocytes undergo cell-cycle arrest and polyploidization. In this issue of *Developmental Cell*, Han et al. (2020) show that Lamin B2, a nuclear lamina filament supporting cardiomyocyte karyokinesis, also facilitates cell division and cardiac regeneration.

Heart attacks remain a significant detriment to human health, largely due to the inability of adult mammals to recover lost cardiomyocytes after ischemic injury. However, the seminal discovery that neonatal mice transiently possess a robust capacity to regenerate their hearts (Porrello et al., 2011) stimulated much interest in a field focused on the identification of mechanisms to improve the cardiac regenerative response in adult mammals. Mammalian heart regeneration depends on the ability of cardiomyocytes to enter the cell-cycle, divide, and replace damaged heart muscle (Porrello et al., 2013). Interestingly, heart regeneration in newborn mice is lost within the first week after birth, coinciding with cardiomyocyte polyploidization and cell-cycle arrest (Soonpaa et al., 1996). This developmental transition has since been studied by a number of groups, revealing many intrinsic and extrinsic factors regulating cardiomyocyte proliferation (reviewed in Vujic et al., 2020). Despite this progress, the majority of factors identified to date primarily induce cardiomyocyte cell-cycle entry, and still very little is known regarding the molecular and cellular mechanisms required to support cell-cycle progression through mitosis and subsequent cytokinesis. In this issue of *Developmental Cell*, Han and colleagues (2020) illuminate the cellular events necessary to progress through complete cardiomyocyte cell division and highlight the role of Lamin B2 (*Lmnb2*), a component of the nuclear lamina promoting nuclear envelope breakdown, in cardiomyocyte proliferation and heart regeneration (Figure 1).

Cardiomyocyte cell-cycle withdrawal during mammalian postnatal development is tightly coupled with cardiomyocyte polyploidization, an increase in nuclear DNA content. The functional relevance of polyploid cardiomyocytes is still uncertain; however, current evidence suggests that their development is indeed a barrier for cardiac regeneration (González-Rosa et al., 2018; Hirose et al., 2019; Patterson et al., 2017). In rodents, approximately 90% of cardiomyocytes become binucleated (containing two diploid nuclei) within the first weeks after birth (Soonpaa et al., 1996). Binucleated cardiomyocytes are hypothesized to arise through defects in cytokinesis, the final stage in cell division during which cellular cleavage occurs and two daughter cells are formed (Figure 1). In contrast, though the majority (>90%) of adult human cardiomyocytes undergo polyploidization, only 25.5% become binucleated. The majority of the remaining mononucleated cardiomyocytes are tetraploid (reviewed in Derks and Bergmann, 2020). The abundance of mostly mononucleated tetraploid cardiomyocytes in humans

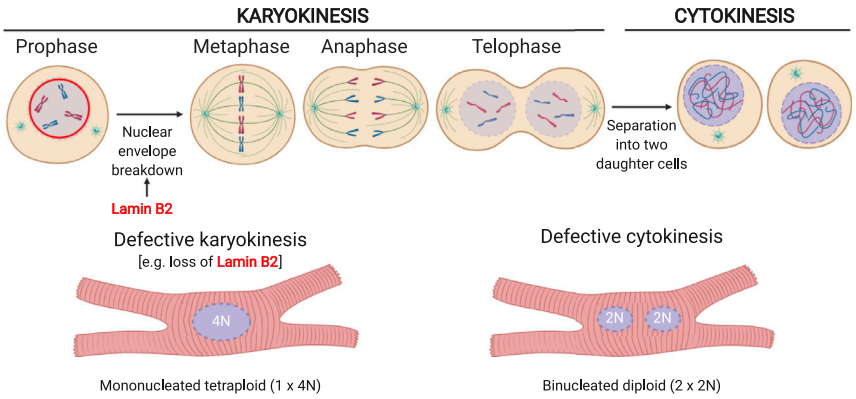

Figure 1. Cell-Cycle Progression and Cardiomyocyte Polyploidization
Lamin B2 supports nuclear envelope breakdown, which is required for transition into metaphase. Defective karyokinesis may result in formation of mononucleated tetraploid cardiomyocytes, whereas cytokinesis failure is hypothesized to generate binucleated cardiomyocytes containing two diploid nuclei. Figure created with BioRender.com.

suggests that an alternative mechanism regulates cardiomyocyte polyploidization, which may include changes in karyokinesis, the division of the cell nucleus during mitosis (Figure 1). Mechanisms that may cause karyokinesis failure in cardiomyocytes have not been defined.

Han and colleagues (2020) set out to explore the cellular mechanisms regulating karyokinesis and its relationship to cardiomyocyte proliferation and regeneration. To do so, the authors used a genetic labeling strategy coupled with fluorescence-activated cell sorting to isolate cycling and non-cycling cardiomyocytes at different developmental stages. They then performed single-cell RNA sequencing and compared the transcriptomes of embryonic cardiomyocytes, capable of complete cell division, to postnatal cardiomyocytes undergoing polyploidization. To enrich for targets potentially involved in karyokinesis, Han et al. (2020) focused on candidates associated with the nuclear membrane and not with cardiomyocyte cell-cycle control. Finally, functional validation experiments directed their attention to Lamin B2 (*Lmnb2*), a nuclear lamin filament protein highly expressed in fetal and early neonatal cardiomyocytes but downregulated during postnatal development. The authors then assessed the function of Lmnb2. *In vitro* loss-of-function and gain-of-function experiments in cultured fetal and neonatal mouse cardiomyocytes demonstrated that *Lmnb2* promotes cardiomyocyte entry into mitosis. Furthermore, *in vivo* cardiomyocyte-specific inactivation of *Lmnb2* decreased the number of cardiomyocytes in mitosis without affecting DNA synthesis, solidifying a role for this protein in mitotic entry. Finally, through detailed analyses of cardiomyocyte cell-cycle progression, Han and colleagues (2020) deduced that *Lmnb2* loss of function impairs the transition from prophase to metaphase.

A key cellular event during prometaphase is nuclear envelope breakdown, which permits microtubules to invade the nuclear space and attach to chromosomes for subsequent alignment and separation during metaphase and anaphase, respectively (Figure 1). Han and colleagues (2020) noted that *Lmnb2* mutant cardiomyocytes possess a higher degree of DNA colocalization with Lamina-associated polypeptide 2 (LAP2), evidence that nuclear envelope breakdown is impaired. Further characterization of tissue-specific *Lmnb2* knockouts revealed a significant increase in nuclear ploidy in both mononucleated and binucleated cardiomyocytes. Cardiomyocyte-specific *Lmnb2* mutant hearts were then evaluated in a neonatal mouse model of cardiac cryoinjury, showing that loss of *Lmnb2* compromises heart function while overexpression of *Lmnb2* improves heart function post-injury and reduces scar formation. Combined, these results suggested that improved nuclear envelope breakdown via *Lmnb2* overexpression may enhance cardiac regenerative capacity. Importantly, Han and colleagues (2020) extended their studies to the human context. Using CRISPR-Cas9 technology, the authors generated *LMNB2* knockout cardiomyocytes derived from human-induced pluripotent stem cells. Consistent with their results in mice, these knockout cell lines exhibited defects in mitotic progression coinciding with a mild increase in polyploid nuclei formation. Furthermore, overexpression of *LMNB2* in cultured human cardiomyocytes decreased the prevalence of polyploid nuclei.

Overall, Han and colleagues (2020) propose a role for Lamin B2 in supporting cardiomyocyte karyokinesis (Figure 1), which facilitates complete cell division and cardiac regeneration. However, it is important to point out that while Lamin B2 protein levels decrease shortly after birth during normal mouse postnatal development, ~90% of murine cardiomyocytes do complete karyokinesis, arresting during cytokinesis and ending up binucleated. One explanation may be that the postnatal reduction of *Lmnb2* expression occurs after the loss of key components of cytokinesis in most rodent cardiomyocytes. A reversed order of these two events in human cardiomyocytes may account for the high prevalence of mononucleated polyploid cardiomyocytes in the adult human heart. Nevertheless, a plausible approach in cardiac regenerative medicine might combine modulation of factors stimulating cardiomyocyte cell-cycle entry with those, such as LMNB2, that enable completion of cardiomyocyte cell division, thereby strongly enhancing heart regeneration in adult mammals.

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