Cardiomyocytes in congenital heart disease: Overcoming cytokinesis failure in tetralogy of Fallot

Olaf Bergmann, MD, PhD

Feature Editor's Introduction—As cardiac surgeons, we may not have the journal *Science Translational Medicine* at the top of our reading lists. Therefore, the associate congenital editors thought it would be appropriate to bring to the attention of the readership the report by Liu and colleagues,¹ which appeared in late 2019 and received comment in *The New England Journal of Medicine* early in 2020.² The report provides preliminary evidence that patients with tetralogy of Fallot demonstrate myocardial cytokinesis failure and that early administration of standard beta-blockade may help restore successful cytokinesis. Cytokinesis failure is typically the replication of nuclear material without cell division resulting in multinucleated cells. Why is this potentially important? In theory, cytokinesis failure leads to a reduction in total myocyte number and a higher percentage of multinucleated myocytes that may respond less favorably to both normal and abnormal stress and growth stimuli. In theory, it may set up a myocardial milieu more prone to heart failure later in life. It appears that we do not know if this failure occurs in the left ventricle, and we do not know if it occurs in other conotruncal abnormalities or even other forms of congenital heart disease. But even if these 2 theoretical suppositions for tetralogy of Fallot are true, then we may have at our disposal a simple, cheap medicine to treat a cellular-level problem that might have implications for long-term cardiac function.

Therefore, the associate editors are pleased and honored to have a world-class expert, Dr Olaf Bergmann, provide commentary on Liu and colleagues' report.³ So put away your scalpels, the debates about type and timing of repair, the concerns about preservation of the valve and infundibulum, and so forth, and turn your attention

Beta-adrenergic signaling regulates cytokinesis in cardiomyocytes through ECT2 in ToF/PS.

CENTRAL MESSAGE

Blockage of beta-adrenergic signaling counteracts cytokinesis failure and promotes cardiomyocyte proliferation through ECT2 upregulation in infants with ToF/PS.

This Invited Expert Opinion provides a perspective on the following papers: 1. *Sci Transl Med*. 2019;11(513):eaaw6419. <https://doi.org/10.1126/scitranslmed.aaw6419>. 2. *N Engl J Med*. 2020;382(3):291-293. <https://doi.org/10.1056/NEJMcibr1913824>.

See Commentaries on pages 1591 and 1592.

to cellular level considerations and enjoy Dr Bergmann's perspective.

Ronald K. Woods, MD, PhD

Tetralogy of Fallot with pulmonary stenosis (ToF/PS) is the most common cyanotic congenital heart disease and involves ventricular septal defect, pulmonary valve stenosis, an overriding aorta, and right ventricular hypertrophy. As the human myocardium is restricted in its ability to generate new cardiomyocytes,^{3,4} the only option to treat this condition is surgical repair within the first years of life to correct for morphological abnormalities. Patients with repaired ToF/PS still show an increased incidence of heart failure in adulthood⁵ and require lifelong specialized medical care.⁶

Cardiomyocyte cell cycle activity is rare in adult heart^{4,7} but can be triggered by pathological conditions such as cardiac infarction and congestive heart disease. However, cell cycle activity in these conditions does not necessarily lead to progression to cytokinesis, the last stage of mitosis

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when the cleavage furrow is formed to separate the new daughter cells from each other, but to premature cell cycle exit, resulting in multinucleation and polyploidy.⁸ Multinucleated cardiomyocytes are thought to enter the cell cycle less frequently and are considered to be less responsive to cell cycle regulators than diploid mononucleated cardiomyocytes.⁹⁻¹¹

In healthy adult human hearts, less than 1% of all cardiomyocytes are newly generated per year.⁴ Although this rate is supposed to be higher in early childhood and infancy,^{4,7} it is debated whether a higher capacity to renew cardiomyocytes is already sufficient to allow for robust heart regeneration in injured human neonatal hearts,¹² as already documented in mouse hearts.¹³

Liu and colleagues¹ investigated whether cardiomyocyte cell cycle activity and cytokinesis are altered in infants with ToF/PS. The authors¹ analyzed biopsies from 12 children with ToF/PS undergoing cardiac repair surgery and compared the level of multinucleation with specimens from subjects with no known heart disease. In a healthy human heart, multinucleation is already completed at birth.⁴ However, in patients with ToF/PS, binucleation continues until the second postnatal month until reaching a level of multinucleated cardiomyocytes of approximately 60%, which is a 2- to 3-fold increase compared with healthy hearts. The high level of multinucleation may depend on genetic alterations in ToF/PS or morphological aberrations and their consequences for heart function.

To directly assess cell cycle activity, the authors¹ performed a pulse-chase experiment in which 1 patient with ToF/PS received the nonradioactive tracer ¹⁵N-thymidine at 1 month of age, and the surgical biopsy was consecutively analyzed at 7 months of age. Seven percent of all mononucleated diploid cardiomyocytes were ¹⁵N-thymidine labeled, indicating that at this developmental stage, cardiomyocytes still have the potential to divide. However, because there was no healthy control included, it is not possible to know whether a similar rate of cardiomyocyte generation would have been seen in an age-matched control sample. Of note, the incorporation of ¹⁵N-thymidine into genomic DNA was identified in 20% of all binucleated cardiomyocytes, indicating that these cardiomyocytes progressed to karyokinesis (division of nuclei) but failed to perform cytokinesis. These experiments suggest that ToF/PS triggers cell cycle activity mainly with cytokinesis failure, resulting in the multinucleation of cardiomyocytes within the first 2 postnatal months. A tempting idea would be to overcome this mitotic block to generate new myocardium for repairing ToF/PS-related defects.

Ect2 has been shown to be a key protein in cardiomyocyte cytokinesis, triggering the constriction of the cleavage furrow.¹⁴ Liu and colleagues¹ showed that *Ect2* is downregulated during the perinatal period when cardiomyocyte proliferation gradually declines and the rate of mitotic failure

increases, resulting in multinucleation in the rodent heart. The overexpression of *Ect2* did not lead to a higher number of cycling neonatal cardiomyocytes per se in vitro, but Liu and colleagues¹ found a 2-fold reduction in the generation of binucleated cardiomyocytes along with a constant rate of S-phase activity, arguing for a specific positive effect on the completion of cytokinesis. Using a transgenic system to lower *Ect2* expression (alpha-MHC-Cre; *Ect2*^{fllox/fllox}), the number of binucleated cardiomyocytes increased 3.2-fold at postnatal day 1 (P1). The total number of cardiomyocytes was reduced by approximately 50%, indicating that *Ect2* is critical for cardiomyocyte duplication in the fetal period of development. With single-cell transcriptional analysis, Liu and colleagues¹ showed that the findings in the mouse cardiomyocytes might also apply to human cardiomyocytes. *ECT2* was also downregulated in cycling cardiomyocytes of patients with ToF/PS. However, because of the unavailability of age-matched human heart tissue from neonates, Liu and colleagues¹ performed this comparison with fetal human tissue from subjects with no heart disease. Thus, one cannot exclude that the observed downregulation of *ECT2* in infants with ToF/PS is only a consequence of the difference in developmental stages being compared but is not necessarily linked to heart disease.

The Hippo-Yes-associated protein (Hippo-Yap) signaling pathway determines organ size by tightly controlling proliferation and apoptosis, and it has been shown to regulate cardiomyocyte proliferation in embryogenesis and in adult rodents.¹⁵⁻¹⁷ Thus, the authors¹ looked for potential interaction of Yap1 (yes-associated protein 1), the terminal effector of the Hippo signaling pathway, and *Ect2*. Liu and colleagues¹ identified Tead (TEA domain family member), a binding partner of Yap1, to have 5 binding sites in the *ECT2* promoter, showing an enhancement of *Ect2* promoter activity. Accordingly, the overexpression of *Tead1* and *Yap1* increased *Ect2* expression and led to a decrease in binucleated cardiomyocytes in vitro, indicating that the Hippo-Yap signaling pathway is involved in the regulation of *Ect2* and cardiomyocyte cytokinesis.

Prompted by evidence that beta-adrenergic signaling can regulate the Hippo-Yap pathway,¹⁸ Liu and colleagues¹ explored with several strategies whether beta-adrenergic signaling affects the expression level of *Ect2*. The treatment of neonatal mouse cardiomyocytes with the beta-blockers propranolol and alprenolol led to a reduction in multinucleation and to an increase in the number of cardiomyocytes, which improved recovery and adverse remodeling in an adult heart infarction model. As mouse neonatal development is difficult to compare with human neonatal hearts, experiments performed with cultured fetal human cardiomyocytes and organotypic heart slices of patients with ToF/PS were conducted, supporting the finding that beta-adrenergic signaling regulates

FIGURE 1. Regulation of cytokinesis in cardiomyocytes in infants with ToF/PS. ECT2 is downregulated in infants with ToF/PS mediated by beta-adrenergic signaling, resulting in cytokinesis failure in cardiomyocytes. Beta-adrenergic blockade rescues this phenotype by reducing beta-adrenergic activity and subsequently increasing the level of ECT2, allowing cytokinesis and cardiomyocyte proliferation. Anatomic drawing of tetralogy of Fallot is adopted from Englert and colleagues.²¹

cytokinesis failure, which can be alleviated by propranolol treatment. However, Liu and colleagues¹ analyzed cytokinesis failure in human specimens only by quantifying the rate of binucleated cardiomyocytes in comparison with mononucleated cardiomyocytes. As human neonatal and adult cardiomyocytes respond to stress and disease conditions with nuclear polyploidization,^{19,20} an increase in the ploidy of mononucleated cardiomyocytes could have been interpreted as newly generated cardiomyocytes, and the rate of successful cytokinesis could have been overestimated.

CONCLUSIONS

The findings by Liu and colleagues¹ showed that cardiomyocytes from patients with ToF/PS showed cell cycle activity with cytokinesis failure mainly occurring within the first 2 postnatal months. This study further suggests that a reduction in beta-adrenergic signaling by beta-blockers rescues cytokinesis failure in cardiomyocytes by upregulating Ect2 (Figure 1 and Table 1). Beta-blockers are widely used in pediatric patients with hypertrophic cardiomyopathy and in heart failure, and the safety profile of beta-blockers in the pediatric population with ToF/PS seems to be favorable.²² Ideally, beta-blocker therapy delivered early in life could overcome cytokinesis failure and generate new cardiomyocytes,

thereby supporting surgical repair adjunctively and reducing the risk of developing heart failure later in life. However, one might ask why functional recovery has not yet been described, because beta-blockers have been used for decades in patients with ToF.²³ Moreover, a randomized study could not show any effect of beta-blockers on heart dysfunction in surgically treated patients with ToF/PS.²⁴ Liu and colleagues¹ suggested that beta-blocker treatment should start as early as possible to obtain the most likely effect on cardiomyocyte generation. Later onset might have only minor or no effect on cardiomyocyte generation. Moreover, the number of rescued cell divisions by completing cytokinesis might not be sufficient to build sustainable amounts of new myocardium. Previous data by Kühn’s group demonstrated that in neonate patients with ToF/PS, the number of mitotic events was dramatically reduced compared with those in healthy age-matched controls.²⁵ Therefore, it might be necessary to complement the proposed strategy with measures to also directly stimulate cardiomyocyte cell cycle activity and proliferation, as previously reported,²⁶ to demonstrate clinical improvement in patients with ToF/PS.

Conflict of Interest Statement

The author reported no conflicts of interest.

TABLE 1. Summary of findings

Cytokinesis failure in postnatal cardiomyocytes is a feature of ToF/PS
Ect2 upregulation increases cytokinesis activity in neonatal cardiomyocytes
The Hippo/YAP pathway regulates Ect2 expression and cardiomyocyte cytokinesis
Beta-blockers increase Ect2 expression and counteract cytokinesis failure in neonatal mice and in ToF/PS cardiomyocytes
Beta-blocker–induced murine cardiomyocyte endowment improves cardiac function after adult myocardial infarction

ToF/PS, Tetralogy of Fallot with pulmonary stenosis.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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