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EDITORIAL

BRAP: a novel regulator of the cardiomyocyte cell cycle controlling both proliferation and survival?

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This editorial refers to 'Control of p21^{Cip} by BRCA1-associated protein is critical for cardiomyocyte cell cycle progression and survival', by C. Volland *et al.*, pp. 592–604.

One of the greatest challenges in heart regeneration is to replace lost myocardium by generating new cardiomyocytes. Recent findings showing that there are no myogenic stem cells in the mammalian heart,[1,2] together with the observation that a small percentage of adult human cardiomyocytes retains the capacity to renew,[3] have shifted the focus to augmenting proliferation of endogenous cardiomyocytes. This spiked a huge interest in understanding cardiomyocyte cell cycle regulation, resulting in several studies showing successful induction of proliferation in adult cardiomyocytes.[4–6]

During developmental and adult stages, mammalian cardiomyocytes go through several stages of growth and decline. Pre-natal growth is characterized by cardiomyocyte proliferation, followed by postnatal hypertrophic growth marked by binucleation and polyploidization, whereas aged hearts suffer from cardiomyocyte apoptosis. All these stages require a specific regulation of the cell cycle and how cardiomyocytes manage to fine-tune this remains poorly understood.

In the present report, Volland *et al.*[7] describe BRCA-1-associated protein (BRAP) as a regulator of the cardiomyocyte cell cycle (*Figure 1*). Previously, BRAP has been shown to act as a key regulator for Ras-to-Erk signalling.[8] Furthermore, a loss of BRAP led to a delay in S phase and stalled G2 phase in neural progenitor cells by controlling p27^{Kip1} degradation through targeting Skp2.[9] Volland *et al.*, BRAP exerted specific effects on cardiomyocytes depending on the stage of cardiac development in mice. BRAP expression levels dropped coinciding with the loss of the proliferative capacity of cardiomyocytes during the neonatal period and declined even further in adults.[7]

To test the hypothesis that BRAP plays a functional role in regulating the cardiomyocyte cell cycle during development, the authors generated several genetic loss- and gain-of-function mouse models. An unconditional knock-out of *Brap* led to embryonic lethality due to developmental retardation in several organ systems. Importantly, next to signs of cardiac congestion, the embryonic heart also showed thinning of trabecular and compact layers of the ventricular myocardium. This suggests that this congestion is a consequence of a lack of contractile tissue. This finding was supported by showing that directed ablation of BRAP in early embryonic cardiomyocytes using Nkx2.5-Cre and Mlc2V-Cre recombinase lines was sufficient to induce embryonic lethality. In contrast, αMHC-Cre/Brap$^{fl/fl}$ mice survived until the first postnatal days, allowing the authors to explore the effect of BRAP expression on cardiomyocyte proliferation in the perinatal period, when cardiomyocytes withdraw gradually from the cell cycle and undergo polyploidization.[10] A specific knock-out of *Brap* caused a reduction of cell cycle activity with a decrease in cardiomyocyte proliferation and binucleation at post-natal Day 4. Next, the authors investigated the effect of *Brap* knock-out in adult hearts, which have a limited renewal capacity. *Brap* was specifically deleted in adult cardiomyocytes using the tamoxifen-inducible αMHC-MerCreMer mouse crossed to the *Brap*$^{fl/fl}$ mouse. In contrast to neonatal mice, the authors found multiple morphological changes comprising a cardiomyopathy phenotype, including the deterioration of cardiac function with increased dilatation. This was accompanied by cardiomyocyte hypertrophy, fibrosis, and increased apoptosis.

To understand how BRAP affects cardiomyocyte cell cycle and survival, a transcriptional analysis of *Brap* knock-out mice was performed. This revealed a decrease in cell cycle promoting genes such as *Cyclin D1* and *Cdk2*, as well as a drastic increase of the cyclin-dependent kinase inhibitor *Cdkn1a* gene (p21^{Cip1}), which is an important negative regulator of the cell cycle and is required for G1/S transition. As BRAP is a negative regulator of nuclear import,[11] its deletion not only increases p21^{Cip1} expression but also leads to an accumulation of p21^{Cip1} in the nucleus. p21^{Cip1} has been described as a regulator for replicative senescence, apoptosis, and DNA damage response depending on its cellular localization.[12] Cytoplasmic p21^{Cip1} protects against apoptosis,[13] whereas nuclear p21^{Cip1} inhibits cell division and growth.[12] Accordingly, the reported increase in nuclear p21^{Cip1} in *Brap* knock-out mice might have altered the nuclear/cytoplasmic ratio of p21^{Cip1}, blocking its anti-apoptotic

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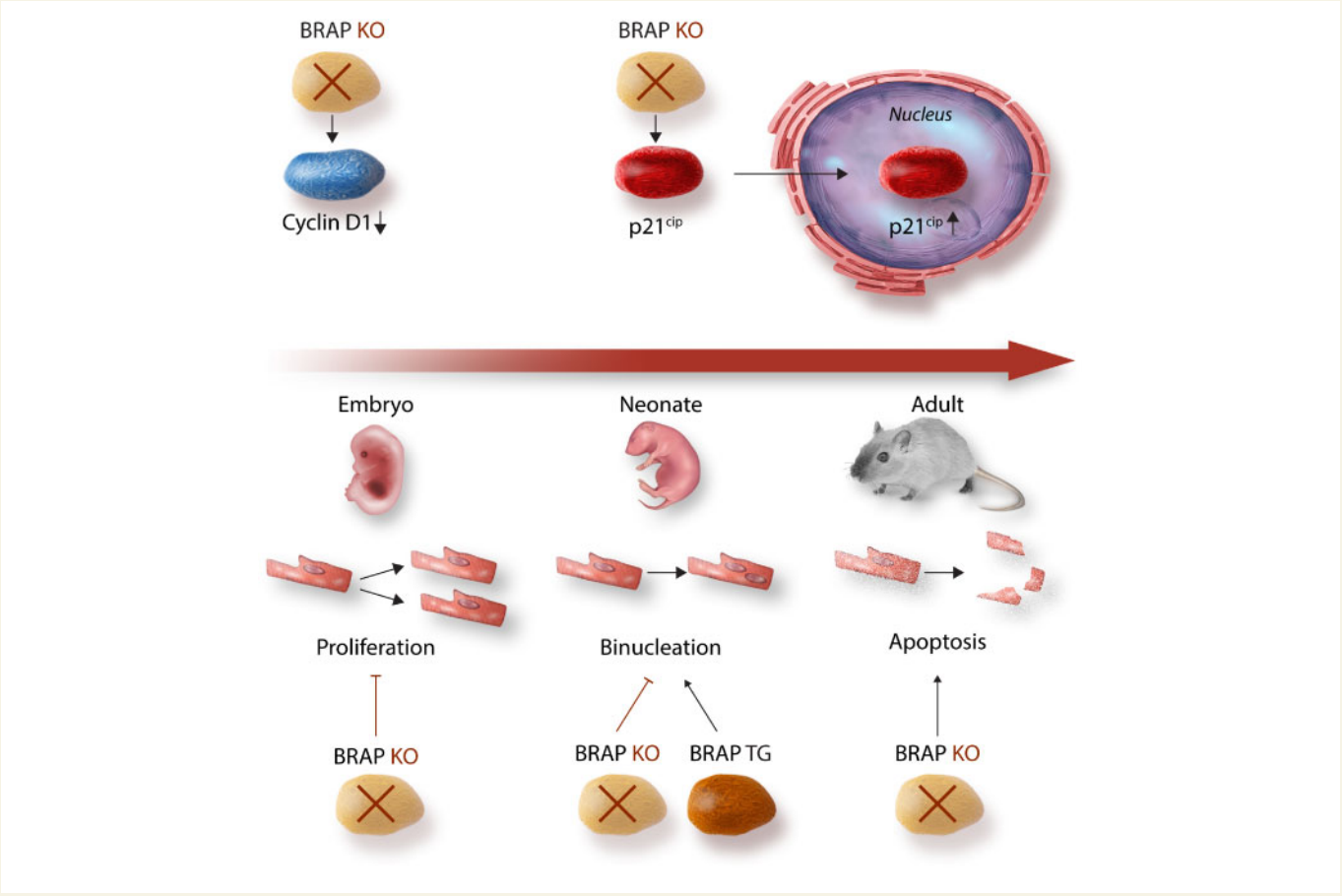

Figure 1 BRAP regulates proliferation, binucleation, and apoptosis at different developmental stages. *Brap* knock-out (KO) decreases expression levels of Cyclin D1 and leads to nuclear accumulation of $p21^{Cip1}$. This causes a cell cycle arrest via suppression of DNA synthesis, thereby inhibition of proliferation and binucleation of cardiomyocytes. In the adult mouse, cardiac deletion of BRAP increased apoptosis, suggesting a role for BRAP in the survival of adult cardiomyocytes. Overexpression of BRAP (TG) leads to an increase in binucleation in neonatal mice.

cytoplasmic effect. Whether the reported cardiomyopathy phenotype is only elicited by the up-regulation of $p21^{Cip1}$, causing an increase in apoptosis, or if the knock-down of *Brap* acts through different pathways needs to be investigated.[14]

Moreover, the authors addressed whether *Brap* overexpression could stimulate cardiomyocyte proliferation. Although cell cycle activity was augmented in neonatal cardiomyocytes, no increase in mitosis was observed, suggesting that overexpression of *Brap* is essential to promote cell cycle entry, but might not be sufficient to stimulate the progression to mitosis and cytokinesis in neonates. Whether continued overexpression of *Brap* in adult hearts would lead to similar effects or even induced cardiomyopathy has not been addressed. In other studies, down-regulation of pocket proteins, including $p21^{Cip1}$, was shown to lead to hypertrophy and eventually to dilated cardiomyopathy.[15] Moreover, it remains to be determined whether BRAP regulation could have a positive effect on cardiac injuries, such as myocardial infarction and induced pressure overload.

The work by Volland *et al.* has generated insights into the role of BRAP on cardiac regeneration. However, future studies will need to decipher its complete role as a potential switch between cell cycle activity in foetal and neonatal cardiomyocyte and survival of adult cardiomyocytes.

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References

1. Elhelaly WM, Cardoso AC, Pereira AHM, Elnawasany A, Ebrahimi S, Nakada Y, Sadek HA. C-kit cells do not significantly contribute to cardiomyogenesis during neonatal heart regeneration. *Circulation* 2019;**139**:559–561.
2. Kretzschmar K, Post Y, Bannier-Helaouet M, Mattiotti A, Drost J, Basak O, Li VSW, van den Born M, Gunst QD, Versteeg D, Kooijman L, van der Elst S, van Es JH, van Rooij E, van den Hoff MJB, Clevers H. Profiling proliferative cells and their progeny in damaged murine hearts. *Proc Natl Acad Sci U S A* 2018;**115**:E12245–E12254.
3. Bergmann O, Zdunek S, Felker A, Salehpour M, Alkass K, Bernard S, Sjostrom SL, Szewczykowska M, Jackowska T, Dos Remedios C, Malm T, Andrä M, Jashari R, Nyengaard JR, Possnert G, Jovinge S, Druid H, Frisén J. Dynamics of cell generation and turnover in the human heart. *Cell* 2015;**161**:1566–1575.
4. Mahmoud AI, Kocabas F, Muralidhar SA, Kimura W, Koura AS, Thet S, Porrello ER, Sadek HA. Meis1 regulates postnatal cardiomyocyte cell cycle arrest. *Nature* 2013;**497**:249–253.
5. Puente BN, Kimura W, Muralidhar SA, Moon J, Amatruda JF, Phelps KL, Grinsfelder D, Rothermel BA, Chen R, Garcia JA, Santos CX, Thet S, Mori E, Kinter MT, Rindler PM, Zacchigna S, Mukherjee S, Chen DJ, Mahmoud AI, Giacca M, Rabinovitch PS,

Aroumougame A, Shah AM, Szweda LI, Sadek HA. The oxygen-rich postnatal environment induces cardiomyocyte cell-cycle arrest through DNA damage response. *Cell* 2014;**157**:565–579.
6. Mohamed TMA, Ang YS, Radzinsky E, Zhou P, Huang Y, Elfenbein A, Foley A, Magnitsky S, Srivastava D. Regulation of cell cycle to stimulate adult cardiomyocyte proliferation and cardiac regeneration. *Cell* 2018;**173**:104.
7. Volland C, Schott P, Didié M, Männer J, Unsöld B, Toischer K, Schmidt C, Urlaub H, Nickels K, Knöll R, Schmidt A, Guan K, Hasenfuß G, Seidler T. Control of $p21^{Cip}$ by BRAP is critical for cardiomyocyte cell cycle progression and survival. *Cardiovasc Res* 2020;**116**:592–604.
8. Lanctot AA, Peng CY, Pawlisz AS, Joksimovic M, Feng Y. Spatially dependent dynamic MAPK modulation by the Nde1-Lis1-Brap complex patterns mammalian CNS. *Dev Cell* 2013;**25**:241–255.
9. Lanctot AA, Guo Y, Le Y, Edens BM, Nowakowski RS, Feng Y. Loss of BRAP results in premature G1/s phase transition and impeded neural progenitor differentiation. *Cell Rep* 2017;**20**:1148–1160.
10. Alkass K, Panula J, Westman M, Wu TD, Guerquin-Kern JL, Bergmann O. No evidence for cardiomyocyte number expansion in preadolescent mice. *Cell* 2015;**163**:1026–1036.
11. Fatima S, Wagstaff KM, Loveland KL, Jans DA. Interactome of the negative regulator of nuclear import BRCA1-binding protein 2. *Sci Rep* 2015;**5**:9459.
12. Georgakilas AG, Martin OA, Bonner WM. p21: a two-faced genome guardian. *Trends Mol Med* 2017;**23**:310–319.
13. Asada M, Yamada T, Ichijo H, Delia D, Miyazono K, Fukumuro K, Mizutani S. Apoptosis inhibitory activity of cytoplasmic p21(Cip1/WAF1) in monocytic differentiation. *EMBO J* 1999;**18**:1223–1234.
14. D'Amora DR, Hu Q, Pizzardi M, Kubiseski TJ. BRAP-2 promotes DNA damage induced germline apoptosis in *C. elegans* through the regulation of SKN-1 and AKT-1. *Cell Death Differ* 2018;**25**:1276–1288.
15. Di Stefano V, Giacca M, Capogrossi MC, Crescenzi M, Martelli F. Knockdown of cyclin-dependent kinase inhibitors induces cardiomyocyte re-entry in the cell cycle. *J Biol Chem* 2011;**286**:8644–8654.