Cell PRESS

Breaking the Silence: Stimulating Proliferation of Adult Cardiomyocytes

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DOI 10.1016/j.devcel.2009.07.022

Several recent findings challenge our view of the heart as a postmitotic organ and suggest that the adult heart has some capacity to regenerate. Bersell et al. in a recent issue of *Cell* report that neuregulin1-mediated activation of ErbB2/4 receptors induces proliferation of adult mononuclear cardiomyocytes.

The once widely held views that mammalian cardiomyocytes stop proliferating early after birth and that the heart itself is a postmitotic organ are now fading (Anversa and Nadal-Ginard, 2002). Several reports using genetic fate-mapping techniques in mice (Hsieh et al., 2007) or assessing the concentration of carbon-14-labeled nucleic acids in human cardiomyocytes (Bergmann et al., 2009) suggest a continuous replacement of myocardial cells during the lifetime of mammals. In principal, replacement of functional, terminally differentiated cardiomyocytes might occur via two different routes: (1) proliferation and differentiation of cardiac or other progenitor cells and (2) proliferation of differentiated cardiomyocytes. These routes need not be mutually exclusive but may be complementing alternatives that contribute to the renewal of the myocardium to different degrees and under different conditions. So far, researchers have concentrated on the detection and functional analysis of cardiac stem cells, a concept that has already led to clinical applications (reviewed by Dimmeler et al., 2008). In contrast, stimulating division and proliferation of mature adult cardiomyocytes has been less popular, in particular since it is rather difficult to activate cell division in mature cardiomyocytes, a fact that might also be responsible for the rare formation of myocardial tumors and the lack of success in obtaining spontaneously immortalized cardiomyocyte cell lines.

Yet, several groups have reported reactivation of the cell cycle by either removing cell cycle inhibitors (e.g., p27) or by overexpressing cell cycle activators such as cyclinD1, E2F2, expression of SV40 large T, dominant interfering p53 and others. The rate of cell cycle activation, however, was always relatively low when applying stringent criteria for the identification of cycling cardiomyocytes. In addition, in most of these studies cardiomyocytes showed changes in gene expression consistent with the possibility that increased proliferation was due to cell cycle reentry of remnants of embryonic cardiomyocytes or precursor cells (reviewed by Rubart and Field, 2006). It therefore came as a surprise that the activation of specific signaling pathways by FGF-1 (together with the inhibition of p38) or periostin (a heterofunctional secreted extracellular matrix protein) could induce up to 1.3% of rat ventricular cardiomyocytes to initiate DNA synthesis and that roughly 50% of these cardiomyocytes then go into cytokinesis. In an article in *Cell*, Bersell et al. (2009) now uncover an additional pathway, in which Neuregulin1 stimulates cardiomyocyte proliferation in the adult heart via activation of the tyrosine kinase receptors erbB2/4. Neuregulin1 and its receptors are well known to be essential for heart development and were thought to control embryonic cardiomyocyte proliferation. In adult cardiomyocytes, Bersell et al. (2009) now demonstrate that Neuregulin1 drives up to 20% of mononuclear (but not binuclear) cardiomyocytes into DNA synthesis. Since less than 10% of the cardiomyoctes in the adult mouse heart are mononucleated, only a small subpopulation responds to Neuregulin1. Furthermore, the proportion of cells that initiate cytokinesis in vivo is even smaller, corresponding to about 0.3% of mononuclear cardiomyocytes (Figure 1A). Still, the fact that these cardiomyocytes are able to proliferate at all is surprising. Using a broad array of different techniques including cell tracing and differential cell labeling, Bersell et al. (2009) demonstrate that activated cardiomyocytes proliferated clonally in vivo without an apparent contribution of cardiac progenitor cells. How do differentiated cardiomyocytes divide? Neuregulin1-treated mononucleated cardiomyocytes appear to disassemble the contractile apparatus by a partial resorption of the sarcomeric Z disks (Figure 1A), which at least superficially resembles the sequential myofibrillar breakdown observed in dividing embryonic cardiomyocytes. Furthermore, neuregulin1, applied 2 weeks after induction of an acute myocardial infarction, improved cardiac function, implying a therapeutic potential. If considered for the treatment for chronic heart failure, however, it remains to be shown that the subset of cardiomyocytes capable of dividing in response to neuregulin1 is still present and responsive in chronic disease states, especially given that ErbB receptors are downregulated during heart failure or pressure overload. Nevertheless, systemic application of recombinant neuregulin1 improved cardiac function in ischemic and nonischemic models of heart failure (Liu et al., 2006) although it remains to be determined whether these therapeutic benefits are due to enhanced cardiomyocyte proliferation or mediated by the various other known effects of neuregulin1 (e.g., effects on survival, cell integrity, and enhanced angiogenesis).

In embryonic heart development, Neuregulin1 is expressed in the endocardium under control of the Notch and Ephrin signaling pathways, where it signals to its cognate receptors erbB2 and erbB4 located in the adjacent myocardium (Figure 1B). Inactivation of Neuregulin1 or its
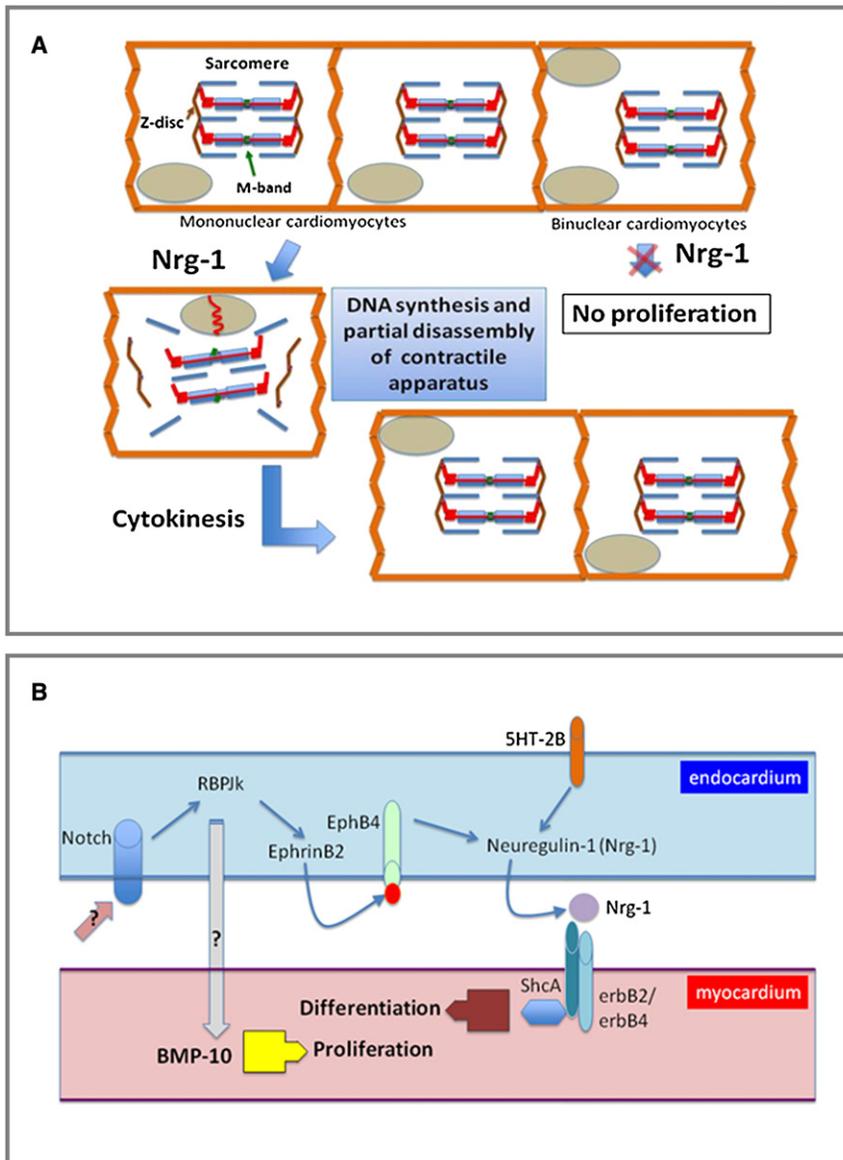
Figure 1. Proposed Model of Neuregulin-1 Actions in Adult Cardiomyocytes and during Embryonic Development
(A) Neuregulin-1 induces partial disassembly of the sarcomeric apparatus and stimulates proliferation of adult cardiomyocytes.
(B) Scheme for the action of neuregulin-1 downstream of notch and the notch-activated RBPjk effector transcription factor during embryonic heart development.

cardiomyocytes. At first glance, such a function would seem to fit to the induction of proliferation of cardiomyocytes by neureglin1 in adults (Bersell et al., 2009) and could explain the effects of neuregulin1 via transient reconstitution of an embryonic signaling pathway. However, more refined experiments disclosed a role of neuregulin1 in the differentiation but not proliferation of trabecular cardiomyocytes during embryonic development. The study found that neuregulin1 could rescue cardiomyocyte differentiation defects caused by a Notch deficiency but could not increase cardiomyocyte proliferation. BMP-10, on the other hand, did promote proliferation (Grego-Bessa et al., 2007).These experiments suggested a model whereby neuregulin1 is released from the endocardium downstream of notch and EphrinB2/EphB4 signaling to activate erbB2/erbB4 heterodimers in the adjacent myocardium (Figure 1B). ErbB2/erbB4, via ShcA, then induce differentiation and maturation of cardiomyocytes while the proliferative stimulus is supplied by BMP-10. Since the Bersell et al. (2009) study shows that neuregulin1 promotes proliferation of adult cardiomyocytes, it appears that neuregulin has distinct effects on embryonic and adult cardiomyocytes.

It has become popular to view regenerative processes in adults as a reiteration of developmental processes. It is becoming clear that this view is an oversimplification. In fact, recent reports emphasize the inadequacy of directly applying knowledge gained from developmental studies to regenerative processes in the adult (Lepper et al., 2009). It seems that the distinct effects of neuregulin1 on embryonic and adult hearts present additional support for this view.

REFERENCES

Anversa, P., and Nadal-Ginard, B. (2002). Nature *415*, 240–243.

Bergmann, O., Bhardwaj, R.D., Bernard, S., Zdunek, S., Barnabe-Heider, F., Walsh, S., Zupicich, J., Alkass, K., Buchholz, B.A., Druid, H., et al. (2009). Science *324*, 98–102.

Bersell, K., Arab, S., Haring, B., and Kuhn, B. (2009). Cell *138*, 257–270.

Dimmeler, S., Burchfield, J., and Zeiher, A.M. (2008). Arterioscler. Thromb. Vasc. Biol. *28*, 208–216.

Grego-Bessa, J., Luna-Zurita, L., del Monte, G., Bolos, V., Melgar, P., Arandilla, A., Garratt, A.N.,

receptors erbB2 and erbB4 results in a lack of trabeculation, a process that involves myocyte proliferation, resulting in abnormally thin myocardium and enlarged ventricles, which causes death before midgestation. Similar phenotypes have been described for mutations in the ShcA gene. ShcA encodes an adaptor protein that transmits signals from the erbB2/erbB4 heterodimer, other tyrosine kinase receptors, and 5HT-2B, a G protein-coupled serotonin receptor. During development 5HT-2B mutants show reduced erbB2 levels in the myocardium, suggesting crosstalk between the two pathways and adding further complexity to cardiomyocyte regulation (for a review see Pentassuglia and Sawyer, 2009). The lack of trabeculation in Neuregulin1 mutants initially suggested that Neuregulin1 might be involved in the control of proliferation or survival of embryonic

Zang, H., Mukouyama, Y.S., Chen, H., et al. (2007). Dev. Cell *12*, 415–429.

Hsieh, P.C., Segers, V.F., Davis, M.E., Macgillivray, C., Gannon, J., Molkentin, J.D., Robbins, J., and Lee, R.T. (2007). Nat. Med. *13*, 970–974.

Lepper, C., Conway, S.J., and Fan, C.M. (2009). Nature *460*, 627–631.

Liu, X., Gu, X., Li, Z., Li, X., Li, H., Chang, J., Chen, P., Jin, J., Xi, B., Chen, D., et al. (2006). J. Am. Coll. Cardiol. *48*, 1438–1447.

Pentassuglia, L., and Sawyer, D.B. (2009). Exp. Cell Res. *315*, 627–637.

Rubart, M., and Field, L.J. (2006). Annu. Rev. Physiol. *68*, 29–49.

Notch Signaling: Linking Delta Endocytosis and Cell Polarity

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DOI 10.1016/j.devcel.2009.07.018

Activation of Notch by its transmembrane ligand Delta requires the E3 ubiquitin ligases Neuralized or Mind bomb and endocytosis of the ubiquitinated ligand. In this issue of *Developmental Cell*, Ossipova et al. show that the polarity regulator PAR-1 phosphorylates Mind bomb, leading to the degradation of Mind bomb and to changes in cell fate due to loss of Notch signaling.

Notch signaling is activated by binding of a transmembrane ligand of the DSL (Delta, Serrate, LAG-2) family to the extracellular domain of the receptor. The binding of the ligand induces a series of proteolytic cleavages of the receptor that ultimately leads to the generation of an intracellular fragment of Notch (Nicd) that enters the nucleus, binds to a DNA binding protein of the CSL [CBF1, Su(H), LAG-1] family and activates transcription (Bray, 2006). A series of recent papers has shown that the activity of DSL ligands depends on their monoubiquitination, which is a prerequisite for endocytosis mediated by the epsin adaptor protein. It is not fully understood why endocytosis of DSL proteins is required for their activity. One explanation may be that endocytosis exerts a mechanical force on the extracellular domain of Notch bound to its ligand while it is endocytosed in the adjacent cell. This force may in turn facilitate the shedding of the extracellular domain of Notch, which would promote subsequent proteolytic cleavages of the receptor. Alternatively, ubiquitination could lead to the sequestration of the ligand to a specific endocytic compartment in which the ligand undergoes further modification (Bray, 2006).

The ubiquitination of DSL ligands depends on the E3 ubiquitin ligases Neu-

ralized and Mind bomb, which are evolutionarily conserved and function in a tissue-specific manner. Loss-of-function of both ligases results in accumulation of DSL ligands at the cell surface and prevents their endocytosis and activation (Le Borgne et al., 2005). These findings clearly showed that Neuralized and Mind bomb are essential for Notch signaling and raised the question of how these proteins are regulated. The work by Ossipova et al. (2009) now shows convincingly that Mind bomb is regulated at the posttranscriptional level by the kinase PAR-1, which so far has been studied mostly with respect to its function in the control of cell polarity. In coimmunoprecipitation experiments followed by mass spectrometry analysis, PAR-1 was identified as a binding partner of Mind bomb. Phosphorylation of Mind bomb by PAR-1 at a conserved serine residue led to its degradation, which was dependent on its E3 ligase activity and the proteasome. Consistent with this finding, overexpression of PAR-1 in tissue culture cells led to reduced ubiquitination of Delta-like 1 (Dll1).

The activity and subcellular localization of PAR-1 is regulated by atypical protein kinase C (aPKC), a core component of the PAR-3/PAR-6/aPKC complex, which

is a central regulator of cell polarity in many cell types throughout the animal kingdom (Suzuki and Ohno, 2006). aPKC directly phosphorylates PAR-1 at a conserved threonine residue, leading to its dissociation from the cortex and inhibition of its kinase activity (Hurov et al., 2004). By this mechanism, aPKC restricts the subcellular localization and activity of PAR-1 to cortical regions where aPKC is not present. As a consequence, aPKC and PAR-1 are localized in a mutually exclusive manner in many polarized cell types, including the *C. elegans* zygote, *Drosophila* neural stem cells, and mammalian and *Drosophila* epithelial cells (Figure 1A) (Suzuki and Ohno, 2006; Krahn et al., 2009). The polarized localization of aPKC and PAR-1 leads to the segregation of both proteins into separate daughter cells upon asymmetric cell division. There is now a solid body of evidence showing that aPKC and PAR-1 are not only required for polarization of the cell prior to asymmetric cell division, but that they also have profound effects on the cell fate of the resulting daughter cells. This has been shown for *Drosophila* larval neuroblasts, where the cell that inherits aPKC will maintain a progenitor fate instead of entering the pathway to terminal neuronal differentiation (Lee et al., 2006).