

Cardiomyocyte proliferation: paving the way for cardiac regenerative medicine without stem cell transplantation

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This editorial refers to ‘TWEAK is a positive regulator of cardiomyocyte proliferation’ by T. Novoyatleva et al., pp. 681–690, this issue.

Acute myocardial infarction and inherited cardiomyopathies, due to extensive loss of cardiomyocytes, may progress towards heart failure despite revascularization procedures and pharmacological treatments. Initial studies supported the concept that bone marrow cells may hold the promise of rebuilding the injured heart from its component elements, offering a valid alternative to the ultimate resort of heart transplantation.¹ However, stem cell biology turned out to be considerably more complex than initially expected. Different stem cell populations, including mesenchymal stem cells, adipose- and amniotic fluid-derived stem cells, and cardiac-resident stem cells have been progressively characterized. It is also evident that growth factor secretion from transplanted stem cells may activate angiogenic, antiapoptotic, and antifibrotic paracrine patterning, playing a major role in cardiac repair.^{2,3} Stem cell growth and differentiation may be subjected to autocrine regulation by secreted growth factors or it may be orchestrated in an intracrine fashion by growth regulatory peptides acting within their cell of synthesis through nuclear receptors and signaling.^{4,5} On the basis of these intriguing dynamics, it is not surprising that multiple randomized clinical trials with autologous bone marrow cells in acute myocardial infarction yielded modest, transient, or no improvement in cardiac performance.^{6–9} Indeed, cardiovascular commitment and secretion of trophic mediators are extremely low-yield processes in both adult and embryonic stem cells. To this end, cell-based phenotypic- and pathway-specific screens of natural and synthetic compounds are on the way to providing a number of molecules that can achieve selective control of stem cell growth and differentiation.^{3,10}

A different approach in providing a source of new cardiomyocytes may be based on the idea of coaxing adult cardiomyocytes to reenter the cell cycle. Enabling proliferation in these cells may represent an alternative area of inquiry in cardiac reparative/regenerative medicine and may also involve a paradigm shift in the current view(s) of cardiac cell therapy. Compelling evidence indicates that adult cardiomyocytes still harbour signalling circuits capable of efficiently resuming their proliferative potential,^{11,12} suggesting that stem cells may not represent the only tool to afford a regenerative medicine for the heart. Within this context, based on the finding that induction of cardiomyocyte proliferation *in vivo* may promote heart regeneration, in this issue of *Cardiovascular Research*, Novoyatleva et al.¹³ aimed at investigating the effects elicited by TNF-related weak inducer of apoptosis (TWEAK) on postnatal rat cardiomyocytes. TWEAK, a member of the TNF- α family regulates proliferation in multiple cell types including liver oval cells, salivary epithelial cells, skeletal muscle myoblasts, kidney mesangial cells, podocytes, and tubular cells.¹⁴

TWEAK is produced as a type II transmembrane glycoprotein and is processed into a 156-amino acid soluble cytokine. Its biological processes involve inflammation, angiogenesis, and cell survival and are mediated through the fibroblast growth factor-inducible molecule 14 (FN14) receptor, a finely tuned, inducible receptor encompassing multiple downstream signalling cascades.¹⁴

Novoyatleva et al.¹³ report that stimulation of neonatal rat cardiomyocytes with TWEAK recombinant protein resulted in a remarkable, dose-dependent increase in DNA synthesis. TWEAK also increased the expression of the proliferative markers cyclin D2 and Ki67, whereas down-regulating the cell cycle inhibitor p27KIP1. The pro-mitotic action of TWEAK was further supported by the observation that it enhanced the number of H3P-positive cardiomyocytes. Evidence that TWEAK led to effective cardiomyocyte division was provided by the finding that the cytokine also increased the

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number of myocardial cells concomitantly stained for Aurora B, a marker of the central spindle and the mid-body, and Troponin I. Loss-of-function experiments using the ITEM-2 antibody (blocking the interaction between TWEAK and FN14) or FN14 siRNA revealed that re-induction of proliferation was dependent on FN14 signalling. Assessment of the phosphorylated form of targeted kinases and the use of specific kinase inhibitors indicated that the TWEAK/FN14-mediated patterning involved the activation of ERK and PI3K as well as inhibition of GSK-3 β , which in turn led to stabilization and accumulation of total β -catenin and accumulation of dephosphorylated β -catenin in the nucleus. TWEAK did not affect proliferation of adult rat cardiomyocytes. This different behaviour resulted from progressive down-regulation of FN14 gene and protein expression after birth. Accordingly, adenoviral expression of FN14 enabled efficient induction of cell cycle reentry in adult cardiomyocytes after TWEAK stimulation. To this end, overexpression of FN14 receptor alone induced DNA synthesis in adult cardiomyocytes, owing to the presence of endogenous TWEAK protein in these cells.

On the whole, these findings may have relevant biomedical implications in cardiac regenerative medicine. In fact, the clinical use of stem cells will be hampered in the near future by a number of inter-related challenges, including: (i) high-throughput bioprocess development and improved downstream processing problems; (ii) significant modification, improvement, and re-testing of current strategies of stem cell culturing and cardiovascular commitment complying with all standards of Good Manufacturing Practice (GMP); (iii) analytical methodologies for control of GMP bioprocessing and differentiation efficiencies. Therefore, the timing for cell culture and expansion within a GMP setting will involve a substantial delay (several weeks) in autologous stem cell transplantation after the acute phase of a heart attack. On the contrary, efficient induction of proliferation in resident adult cardiomyocytes may represent a rapid, first aid to rescue a failing heart. Direct functional approaches should then be designed in experimental models of myocardial infarction or failure to assess whether activation of TWEAK signalling *in vivo* may contribute to replenish lost cardiomyocytes and improve myocardial performance. Further efforts should also attempt to identify chemical compounds that functionally replace viral vector-mediated overexpression of FN14 in adult cardiomyocytes.

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References

1. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B *et al*. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001;**410**:701–705.
2. Gnechchi M, He H, Noiseux N, Liang OD, Zhang L, Morello F *et al*. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. *FASEB J* 2006;**20**:661–669.
3. Ventura C, Cantoni S, Bianchi F, Lionetti V, Cavallini C, Scarlata I *et al*. Hyaluronan mixed esters of butyric and retinoic acid drive cardiac and endothelial fate in term placenta human mesenchymal stem cells and enhance cardiac repair in infarcted rat hearts. *J Biol Chem* 2007;**282**:14243–14252.
4. Ventura C, Branzi A. Autocrine and intracrine signaling for cardiogenesis in embryonic stem cells: a clue for the development of novel differentiating agents. *Handb Exp Pharmacol* 2006;**174**:123–146.
5. Ventura C, Zinellu E, Maninchedda E, Maioli M. Dynorphin B is an agonist of nuclear opioid receptors coupling nuclear protein kinase C activation to the transcription of cardiogenic genes in GTR1 embryonic stem cells. *Circ Res* 2003;**92**:623–629.
6. Cleland JG, Freemantle N, Coletta AP, Clark AL. Clinical trials update from the American Heart Association: REPAIR-AMI, ASTAMI, JELIS, MEGA, REVIVE-II, SURVIVE, and PROACTIVE. *Eur J Heart Fail* 2006;**8**:105–110.
7. Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Desmet W *et al*. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *Lancet* 2006;**367**:113–121.
8. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Forfang K, ASTAMI investigators. Autologous stem cell transplantation in acute myocardial infarction: The ASTAMI randomized controlled trial. Intracoronary transplantation of autologous mononuclear bone marrow cells, study design and safety aspects. *Scand Cardiovasc J* 2005;**39**:150–158.
9. Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S *et al*. Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized, controlled BOOST (BOne marrOW transfer to enhance ST-elevation infarct regeneration) trial. *Circulation* 2006;**113**:1287–1294.
10. Zhu S, Wurdak H, Wang J, Lyssiotis CA, Peters EC, Cho CY *et al*. A small molecule primes embryonic stem cells for differentiation. *Cell Stem Cell* 2009;**4**:416–426.
11. Bersell K, Arab S, Haring B, Kuhn B. Neuregulin1/ErbB4 signaling induces cardiomyocyte proliferation and repair of heart injury. *Cell* 2009;**138**:257–270.
12. Kuhn B, Del Monte F, Hajjar RJ, Chang YS, Lebeche D, Arab S *et al*. Periostin induces proliferation of differentiated cardiomyocytes and promotes cardiac repair. *Nat Med* 2007;**13**:962–969.
13. Novoyatleva T, Diehl F, van Amerongen MJ, Patra C, Ferrazzi F, Bellazzi R *et al*. TWEAK is a positive regulator of cardiomyocyte proliferation. *Cardiovasc Res* 2010;**85**:681–690.
14. Winkles JA. The TWEAK-Fn14 cytokine-receptor axis: discovery, biology and therapeutic targeting. *Nat Rev Drug Discov* 2008;**7**:411–425.