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Human Cardiospheres Are a Source of Stem Cells with Cardiomyogenic Potential

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Several laboratories have shown that collection of adult cardiac stem cells can be grown directly from myocardial tissue [1–7]. Expanded cells are multipotent and clonogenic [3–5]. Spherical aggregates of cells grown from heart biopsies, termed cardiospheres, self-assemble in suspension culture and are enriched in stemness [1, 6, 7]. Andersen et al., in work on neonatal murine heart tissue, used novel culture methods as a basis to question the cardiogenic potential of cardiosphere-cultured stem cells [8]. We contend that their sweeping conclusions are not applicable to studies using established methods [1, 2, 6, 7].

To confirm the cardiogenic nature of human cardiosphere-derived cells (CDCs) in vivo, we evaluated the ability of CDCs to engraft and form new cardiomyocytes after transplantation into infarcted severe combined immunodeficiency (SCID) mice. Immunostaining for human nuclear antigen (1:50, Chemicon MAB1281, Millipore, Billerica, MA, http://www.millipore.com/) and lentivirally mediated green fluorescent protein (GFP) or β-galactosidase labeling were used to track cells after injection. Although the majority of CDCs could be found throughout the infarct (57% $\pm$ 3% of the total engrafted) and the immediate border zone (30% $\pm$ 5%), stable engraftment also existed in the remote myocardium (13% $\pm$ 3%). Figure 1A, 1B shows GFP-labeled CDCs within the border and infarct zones. Counterstaining for cardiac troponin I (cTnI; Chemicon MAB3150) demonstrates that many CDCs have differentiated into cardiomyocytes. As shown in Figure 1, portions of the mouse heart were reconstituted by human CDCs (Fig. 1A), while CDC-derived cardiomyocytes in the dense central infarct zone remained small with little cTnI expression in their cytoplasm (Fig. 1B). Human nuclear antigen expression also demonstrates differentiated cardiomyocytes, derived from transplanted human CDCs, within the border zone (Fig. 1C). These human-derived cells express Cx43, suggesting, but not proving, functional integration within the infarcted tissue (Fig. 1D). Such functional integration has, however, been rigorously documented in vitro with myocyte-CDC coculture [7]. Further, β-galactosidase-labeled CDCs expressing markers of endothelial (Von Willebrand factor [vWF]; Fig. 1E) and smooth muscle (alpha smooth muscle actin[αSMA]; Fig. 1F) lineages highlight the in vivo multilineage potential of CDCs.

These results challenge the conclusions of Andersen et al. by demonstrating in vivo cardiomyogenesis of transplanted cells cultured using established cardiosphere methods. Our laboratory and others have shown that CDCs also decrease infarct size and improve myocardial function [1, 7], with the different subpopulations within CDCs acting synergistically to improve myocardial function [9]. Although cardiac differentiation of transplanted CDCs does occur consistently, long-term persistence of transplanted CDCs is relatively low [10], with important contributions of paracrine effects (both tissue preservation and recruitment of endogenous regeneration) to the functional benefit of CDC transplantation [11].

In this light, Andersen et al. [8] may have overstated the relevance of their findings, considering that their work made use of several unprecedented experimental procedures with novel experimental findings that occurred exclusively when neonatal rat heart was used as a tissue source. These investigators stored the myocardial tissue before processing in a novel buffer, potentially damaging the cells of interest; performed prolonged enzymatic digestion to collect cells from explant culture, possibly favoring the survival of contaminating cells; and finally, failed to characterize their cells in vivo [12]. If the authors seek to convincingly critique the cardiosphere approach, they would be well advised to reproduce established methods. As such, their work is unrelated to adult cardiosphere culture and, as performed, provides no justification for the indictment of the clinical utility of cardiospheres or CDCs, which are already being tested in human subjects [13].

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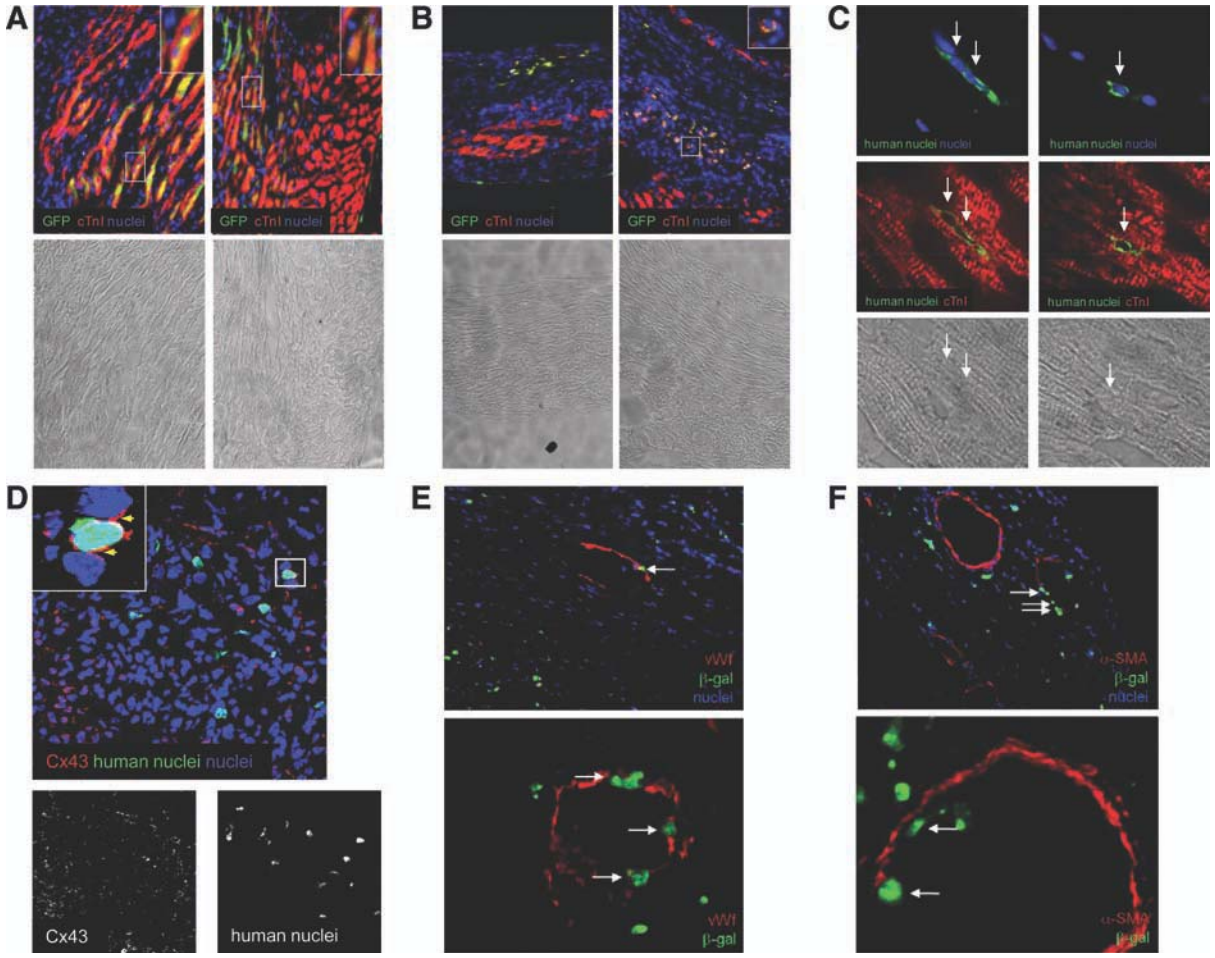

Figure 1. Cardiogenic differentiation of transplanted cells cultured from human biopsies using the cardiosphere method. Human cardiosphere-derived cells (CDCs) were injected into severe combined immunodeficiency mice at the time of myocardial infarction. **(A):** Example images of the infarct border zone demonstrating engraftment and differentiation of lentiviral GFP-labeled CDCs 6 weeks after injection. **(B):** Example images of the central scar demonstrating rounded GFP-labeled CDCs weakly expressing markers of cardiac differentiation 6 weeks after injection. **(C):** Examples of unlabeled human CDCs within the infarct (left image) and border zone (right image) 3 weeks after injection. **(D):** Example of human-derived cardiomyocytes within the infarct border zone expressing Cx43 1 week post-transplantation. **(E):** Example image of β-galactosidase-labeled human CDCs differentiating into smooth muscle cells 3 weeks after injection. **(F):** Example image of β-galactosidase-labeled human CDCs differentiating into endothelial cells 3 weeks after injection. Abbreviations: cTnI, cardiac troponin I; GFP, green fluorescent protein; vWF, Von Willebrand factor; α-SMA, alpha smooth muscle actin.

Inc.) that licenses techniques used to manufacture cardiac stem cells. Dr. Rachel Ruckdeschel Smith is employed by Capricor, Inc. The remaining coauthors have nothing to disclose.

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