Letter Regarding the Article by Xue et al, “Functional Integration of Electrically Active Cardiac Derivatives From Genetically Engineered Human Embryonic Stem Cells With Quiescent Recipient Ventricular Cardiomyocytes”

To the Editor:

The article by Xue et al1 on human embryonic stem cell-derived pacemakers illustrates that embryonic stem cells differentiated into spontaneously beating cardiocytes may function as biological pacemakers and mentions a potential limitation: The intrinsic pacemaker rate was slower than desirable. They suggest that incorporating HCN pacemaker channel genes might achieve more desirable rates, an idea consistent with our published results using HCN2 in gene- and adult human mesenchymal stem cell (hMSC)-based therapies.2–4

However, certain of the comments by Xue et al misinterpret our own work on HCN-loaded hMSCs. They state that “. . .these modified, undifferentiated, human mesenchymal stem cells are incapable of pacing quiescent cells because the former are neither electrically active nor genuine cardiac cells” (p 19). This statement suggests a misunderstanding of the rationale and underlying biophysics of the hMSC experiments. In fact, generation of pacemaker activity does not require delivery of an “excitable differentiated cardiac cell,” but only that the delivered cell (1) carry sufficient pacemaker current and (2) make gap junctions; thus, the hMSC-myocyte pair should behave as a pacemaker unit entirely equivalent to a single heart cell with substantial i. We clearly demonstrated both functions for our genetically engineered hMSCs.2,5

We further take issue with the statement by Xue et al that biological pacemakers must pace quiescent cells. Extrapolating this to human disease implies that biological pacemakers should initiate activity in non-beating hearts. Yet, this is not what an electronic or a biological pacemaker is expected to do clinically. Rather, both types of pacemaker should initiate activity in hearts beating perilously slowly, putting patients at risk of devastating arrhythmia, syncope, or death. Therefore, although our HCN-loaded hMSC biological pacemaker can pace quiescent cells, there is much to be said conceptually for a biological pacemaker that increases dangerously slow heart rates.

Xue et al also criticize our earliest in situ gene therapy publication.2 They imply a deficiency in this study derived from our use of vagal suppression to eliminate sinoatrial pacemaker function. Given that in the absence of atrioventricular block and bundle branch insertion of HCN2, the requirement for vagal suppression was overdriven by the sinoatrial node, the requirement for vagal suppression is not relevant to examination of therapeutic potential. Moreover, in subsequent studies, we showed that during vagal stimulation, an HCN2-based biological pacemaker inserted locally into a bundle branch is functional,3 and that in chronic atrioventricular block and bundle branch insertion of HCN2, physiologically relevant rates are achieved.6

In closing, we welcome criticism and discourse but believe it essential that this be based on an accurate interpretation of the subject matter under discussion.

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Response

We agree that Rosen et al have elegantly provided extensive proof-of-concept data that HCN2-transduced human mesenchymal stem cells (hMSCs), although otherwise quiescent by themselves, could facilitate pacing. Our approaches and designs for studying and engineering pacemaker activity, however, are different: We differentiate pluripotent human embryonic stem cells (hESCs) into an ex vivo sinoatrial (SA) node-like structure, which is capable of generating electrical rhythms on its own accord, for in vivo transplantation and other experiments.2 Unlike native nodal pacemaker cells, transduced hMSCs are indeed neither electrically-active nor genuine cardiac cells. The electrophysiological properties of hMSCs are also somewhat heterogeneous,3 a common property that undifferentiated mouse and hESCs also appear to share.4 It is uncertain over time whether hMSCs after transplantation will further differentiate into cardiac or even non-cardiac cells with electrical properties different from those of pre-transplanted cells.

Although cardiac derivatives from hESCs, genetically-modified or not, are genuine heart cells,5 we likewise do not yet know how long and to what extent electrically active hESC-derived cardiomyocytes will continue to retain their “nodal-like” phenotype once transplanted into the in vivo ventricular or atrial environment. As Rosen et al correctly pointed out, the induced rate observed in our reported experiment was slow. This could be attributed to the above reason. Alternatively, the graft size, transplantation site, purity of nodal cells in the graft, time course for maturation, etc, are other contributing factors that also need to be considered. Our naïve working hypothesis, which remains to be experimentally tested, is that ex vivo overexpression of particular HCN channels can perhaps somehow compensate for the reduced efficacy after transplantation by (1) boosting the basal firing frequency in vitro and/or (2) increasing the percentage of nodal (or nodal-like) cardiomyocytes. This strategy of HCN manipulations, however, is somewhat complicated by the
complex molecular identity of native If (as the 4 isoforms, whose expression levels differ depending on the cell type and species, can coassemble with one another\textsuperscript{7}) and the presence of such ionic components as IK\textsubscript{1} that can influence the ultimate functional effects of If per se.\textsuperscript{8} Protein engineering may be useful for custom-tailoring their biophysical properties for achieving specific outcomes.\textsuperscript{9,10}

To unleash and study the induced pacing activity of our transplanted human cells, we needed to surgically remove the atria and cryoablate the atrioventricular node because the induced rate by human cells is overridden (by the intrinsically faster native guinea pig SA and atrioventricular nodes).\textsuperscript{2} Rosen et al chose to perform vagal stimulation in dogs. Although not explicitly stated, we hinted that future in vivo experiments of this kind can perhaps be facilitated by developing a novel animal model that enables the efficacy of cell transplantation or gene transfer approaches be more specifically investigated, free from undesirable effects due to extensive surgical manipulations, escape rhythms, etc.

Given that the native SA node is such a complex structure consisting of a gradient of nodal pacemaker cells with a range of phenotypic properties, etc.\textsuperscript{11} it is likely that all of the above-mentioned factors will need to be carefully taken into consideration for future experiments designed to improve our understanding of the basis of pacing, and to bioengineer a surrogate SA node.

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