Quantifying Chimeric Cardiomyocytes

To the Editor:

We have read with interest the recently reported results of Deb et al., in which the authors claim that for the first time, human bone marrow was established as a source of extracardiac progenitor cells capable of de novo cardiomyocyte formation. In this context, we would like to refer to a previously published paper from our laboratory reporting that gender-mismatched transplantation of unmanipulated bone marrow from female donors generated chimeric cardiomyocytes and endothelial cells in cadaver hearts of 5 male patients. Controversy arises concerning quantification of this phenomenon, which is of great clinical importance concerning myocardial regeneration and revascularization. Recognition of chimeric cardiomyocytes in sections of heart muscle tissue is hampered by several obstacles, among which are the technique of fluorescence in situ hybridization (FISH), method of counting, influence of a certain section plane, and autolytic changes in postmortem tissue. Unfortunately, in the study by Deb et al., these points were only marginally addressed. Apparently, to identify male chimeric cardiomyocytes, only probes labeling the Y chromosome were used. The statement that in cardiomyocytes of 4 female controls no Y chromosome was shown should have been cross-checked by the demonstration of the X chromosomes. Moreover, no male control hearts were used in this study; therefore, the reported detection sensitivity level for the Y chromosome (45%) is not determinable. By applying FISH in normal human male cadaver hearts, other authors have found a value ranging from 44% to 53%.

Failure to recognize positive signals in cardiomyocytes may be significantly influenced by the plane of section and the preservation of tissue. Immunostaining may be positive in autolytic areas, where detection of proper FISH signals is not possible. For this reason, the corresponding figures for chimeric heart muscle cells have to be corrected accordingly. As already noted by the authors in their discussion, there is a significant discrepancy between the quantity recorded for chimeric cardiomyocytes with an almost 30-fold increased value in our material (6.4% versus 0.23%). This difference is explainable because we applied dual-color FISH and explicitly counted cardiomyocytes that displayed 2 distinctive signals (XX/XY) to avoid all these pitfalls.

Finally, the question of specific immunostaining arises. This is certainly a requirement for an investigator not familiar with histology. For an experienced pathologist, however, no problem exists in differentiating an interstitial cell from a cardiomyocyte or a rarely occurring overlapping of nuclei in properly processed thin tissue sections.

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Response

We thank Dr Kvasnicka and colleagues for their interest in our article and appreciate their comments. We agree that technical methods used for detection of chimeric cardiomyocytes such as the specific FISH protocol, type of microscopy used, number and method of counting nuclei, and quality of tissue sections analyzed are important factors that could affect the degree of chimerism observed. To address such technical issues, we adopted rigorous criteria for identifying chimeric cardiomyocytes as previously described and for identifying formation of pseudonuclei and cell fusion events.

The main critique of our findings by Kvasnicka and colleagues appears to be the lack of X chromosome data in our study. However, if one reads the methodology outlined in our study, it clearly states that X and Y chromosome analysis was used. We did not present X chromosome data in figure format because of space constraints. Moreover, our detection efficiency for XX chromosome detection in normal female myocardium ranged from 40% to 55%, which is consistent with our previously reported efficiency for XX chromosome detection in the vessel wall. In both tissues, the sensitivity of our FISH technique matches that reported by other investigators.

However, a more likely explanation for the observed chimerism differences in our two studies relates to correct identification of chimeric cells as true cardiomyocytes. Notwithstanding autolytic changes, concomitant immunostaining of a cardiomyocyte with cardiac-specific antibodies definitively identifies a cell as a cardiomyocyte and removes an element of human subjectivity inherent in morphological identification of unstained cells. Moreover, additional costaining for laminin allows for the proper delineation of pseudonuclei, the identification of which is difficult for even an experienced pathologist. This issue of pseudonuclei was previously addressed in depth in this journal. Indeed, in our experience, the absence of laminin costaining causes serious overestimation of “chimeric cardiomyocytes.”

Finally, our study differs from that of Thiele et al. with respect to other variables such as mean transplantation-death interval and number of nuclei counted (160 000 vs 1670). We would suggest, therefore, that uniformity in detection techniques, microscopy, number of nuclei counted, and patient profiles must be addressed before comparing degrees of myocardial chimerism estimated in different studies.

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