Adult Stem Cells and Cancer

To the Editor:

Rubio et al. (1) reported in *Cancer Research* that adipose tissue–derived stem cells acquired a transformed phenotype after long-term culture *in vitro*. This article has been cited together with our article published in the same issue (2) as evidence for the malignant transformation potential of stem cells by the news media. Whereas our study showed the dangers of having a continuous high level of telomerase activity during long-term *in vitro* culture of adult stem cells, the study of Rubio et al. employed what seemed to be "normal" cells. We have previously cultured several strains of normal human bone marrow–derived mesenchymal stem cells and we have not observed "immortalization" of the cells (3). Unfortunately, the authors refer to our cells as another example of the human adipose-derived MSC, which is not correct. The differences between our study and the study by Rubio et al., therefore, need clarification because they may have an important effect on the stem cell field. It is possible that the donors employed by Rubio et al. were harboring a disease and the clinical data related to these donors need to be provided as well as the reason for undergoing the surgical procedure. Furthermore, growth curves of the individual cell lines established should be provided and the authors should provide data showing at which population doubling level the cells entered crisis. Finally, the high percentage of the transformation events and the similarity between the cell lines described require proof that the cells that formed the tumors were the cells present at the beginning of the culture by using DNA fingerprinting. These issues are important to resolve because they are important for the stem cell community as a whole.

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In Response:

In our previous paper (1), we referred to Serakinci's work (2) as an example of human adipose-derived mesenchymal stem cells when they really worked with bone marrow–derived mesenchymal stem cells. We apologize for the confusion that this may have produced among readers.

In response to Dr. Kassem's comments, we would like to clarify that our samples of mesenchymal stem cells were always obtained from appendicitis surgical procedures of patients with no other diseases. In our article, we commented that mesenchymal stem cells reached a senescence phase after 2 months in culture, equivalent to 20 population doublings. After bypassing the senescence phase, all mesenchymal stem cells continued to grow until reaching a crisis phase, 8 months (45 population doublings) after senescence bypass. This growth curve is the rule when working with mesenchymal stem cells. The growth curve reached a plateau for ~2 weeks during the senescence phase.

Dr. Kassem cautions about the possible existence of contamination (another cell line) of the samples that became transformed. Because this was a major concern for us, we took some safety checkpoints. Anytime transformation was confirmed, we recultured an aliquot of the original mesenchymal stem cells, frozen at the second initial passing. We then maintained these samples under the culture conditions described in the paper and obtained the same results: transformation among aliquots of samples that had already became tumorigenic and death among aliquots of samples that never did. This fact points to some genetic susceptibility in the spontaneous transformation of mesenchymal stem cells. In addition, we transduced mesenchymal stem cells with retroviral vectors and enhanced green fluorescent protein expression was maintained both in the pretransformed and in the posttransformed mesenchymal stem cells. Another important point was the fact that the same type of malignant transformation was observed in human as well as in murine adipose-derived mesenchymal stem cells. Taken together, these data support the idea that spontaneous transformation of adipose-derived mesenchymal stem cells had occurred *in vitro*. We also think that the transformation capacity of mesenchymal stem cells is not universal, but there is a still unknown susceptibility in some, but not all, mesenchymal stem cell samples. We agree with Dr. Kassem that this is an exciting field of research, with clear importance when thinking in the clinical use of *in vitro*–expanded human mesenchymal stem cells.

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