To the editor:

Chromosomal abnormalities in marrow stromal cells from myelodysplastic syndromes (MDS)

Do marrow stromal cells from patients with hematologic malignancies derive from the leukemic clone? This is a question that has both biologic and clinical relevance, and that, in our opinion, has not yet been answered satisfactorily. In a recent issue of Blood, Ramakrishnan et al addressed this topic, and referred to a previous report by our own group in which we found that in a significant proportion of MDS patients, complex abnormal karyotypes were observed in cultures of adherent stromal cells. In their letter, the authors also referred to studies by other groups in which no clonal abnormalities in MDS stromal cells were observed. Finally, they briefly described their own studies—some of them performed more than 20 years ago—concluding that the stromal component of the marrow microenvironment is not derived from the malignant clone.

Considering the interest that this issue has generated, and based on the communication by Ramakrishnan et al, we would like to make some related comments. In our study, we analyzed the karyotype of pure populations of mesenchymal stem cells (MSCs), devoid of any hematopoietic adherent element; thus, the chromosomal abnormalities observed were present in nonhematopoietic, stromal cells. Such alterations were detected in MSC cultures from 5 (55%) of 9 MDS patients. Although in most cases the alterations found in hematopoietic and stromal cells were different, it is noteworthy that in one case, the chromosomal alterations observed in MSCs were similar to those observed in hematopoietic cells from the same patient. However, and based on our data, we concluded that “our results cannot be considered as evidence that in MDS, MSC are derived from the neoplastic clone.” Indeed, as mentioned in the discussion of our report, a possible explanation for such results is that “the same agent that caused the initial genetic damage in hematopoietic cells (radiation exposure, aromatic compounds, viruses, etc) also affected MSC, although inducing different alterations. As a result of deficiencies in the DNA repair system, centrosome replication and genetic instability, both hematopoietic cells and MSC developed complex, yet different, chromosomal alterations.”

It is important to point out that most studies comparing the genetic integrity of stromal and hematopoietic cells, including the study by Ramakrishnan et al, have used fluorescence in situ hybridization (FISH). One limitation of such a technique is that since the probes used are specific for particular genetic markers, other genetic alterations could not be detected. Thus, the fact that the authors did not find the same genetic marker(s) in stromal and hematopoietic cells is not a conclusive proof that MDS stroma does not derive from the malignant clone. In our study, we analyzed the whole karyotype of both hematopoietic and mesenchymal cells. If we had used FISH, looking for specific alterations present in hematopoietic cells, no genetic alterations in MSCs would have been detected. It is noteworthy, however, that some groups using FISH did find genetic alterations in marrow stromal cells from MDS.

In conclusion, evidence exists that in a proportion of MDS patients, stromal cells bear chromosomal abnormalities that do not necessarily correspond to the same abnormalities found in their hematopoietic counterparts. Whether such alterations have an impact in stromal cell function needs to be determined. Finally, whether stromal cells from MDS patients are derived from the malignant clone is, in our view, still an open question, which needs to be addressed in a more precise and conclusive manner.

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The authors declare no competing financial interests.

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References


Response:

The stromal component of the marrow microenvironment is not derived from the malignant clone in MDS (redux)

We appreciate the issues raised by Flores-Figueroa and Mayani in response to our letter to the editor. Flores-Figueroa et al recently reported the isolation of mesenchymal stem cells (MSCs) from bone marrow of healthy and MDS patients. The authors phenotypically characterized these cells using flow cytometry and immunohistochemistry, and demonstrated by karyotype analysis that MSCs derived from MDS patients contained multiple chromosomal abnormalities.

There are at least 3 possible interpretations for their results: (1) the 5 to 19 karyotypes performed per patient sample were actually done on myeloid cells (CD14+), shown by fluorescence-activated cell sorter (FACS) analysis to contaminate the MSC population; (2) prior chemotherapy plus in vitro expansion introduced chromosome abnormalities in patient MSCs; or (3) the primary disease mechanism predisposes nonhematopoietic tissue to chromosome abnormalities during proliferation.
Of importance, the chromosomal abnormalities seen by Flores-Figueroa et al in the MSCs were different from the ones found in the hematopoietic population.2 These data, along with our previous observation in sex-mismatched hematopoietic stem cell transplant recipients in which we found no evidence of donor-derived stroma 0.15 to 27 years after allogeneic marrow transplantation, strongly suggest that these 2 lineages are derived from distinct stem cells.3,4 The fact that patients with MDS can be cured of their disease by allogeneic peripheral blood stem cell transplantation also suggests that the stroma in MDS is intrinsically normal and that the abnormal function attributed to MDS stroma is the result of interactions between clonal hematopoietic cells and stromal cells.

In our opinion, both reports strongly suggest that the stroma and hematopoietic lineages are distinct: therefore, we conclude that the stromal cells in MDS are not derived from the same transformed stem cell as the hematopoietic clone.

To the editor:

Discrete stem cells: subsets or a continuum?

In a recent issue of Blood, Sieburg et al1 investigated the patterns of clonal repopulation kinetics in a mouse model. The authors demonstrated that the patterns of repopulation observed were limited to a subset of the theoretically possible patterns, implying that the behavior of the cells is patterned and inherited, perhaps through epigenetic modifications.

These data clearly show that hematopoietic stem cells (HSCs) cannot be viewed as “a homogeneous population of cells that respond to the conditions in vivo in a stochastic manner.”1(p2314) However, this description is not an accurate reflection of the continuum models that have recently been proposed.2-4 The problem lies in the authors’ statement that “inherent in the idea of an HSC continuum is the idea that every HSC behavior possible should actually be observable.”1(p2311) In fact, it is implicit in all of these models that stem cells are heterogeneous, not homogeneous, and that differentiation will be “constrained” along certain paths, resulting ultimately in a limited range of cell types.

One mathematic approach to the description of a continuum of potentiality is the concept of a phase space.2-3 This is simply a tool that can be used to describe a complex and continuous system, and there is nothing in the use of such a tool that implies that all points in the conceptual space are equally likely to occur. The mathematics of such a model imply that the behavior of cells at different points within the phase space will be different and may be constrained by both internal and external influences.5

So, why use continuous rather than discrete models? There are 3 principal reasons. The first is that, as Sieburg et al1 acknowledge, the relatively small number of discrete repopulation outcomes that they observed becomes rapidly more complex as additional parameters are considered. While the subset actually observed to be possible/likely may be only a small percentage of the total possibilities, in absolute numbers the observed number of “discrete populations” will continue to increase. Would such an analysis serve to define truly discrete subpopulations of HSCs, or does it rather simply help to define the constraints that exist within a continuous population?

The second reason for modeling stem cells as a continuum is that such modeling permits, and in fact requires, the concept of reversibility. To date all models based on discrete populations have been unidirectional, but there is increasing evidence that reversibility is a reality.

The third reason for using continuous models is that they can be useful tools for examining additional parameters without changing the global paradigm. For example, epigenetic modification will not be constant between donors of different ages. If different patterns were observed in young and old mice, would this imply yet more discrete HSC populations? In a continuous model, such variables can be incorporated as internal variables within the modeled “cell” that constrain or alter the probabilities of different outcomes.

This is not a battle between stochasticists and determinists, but an attempt to find new and potentially more efficient tools to model stem cell behavior, which may ultimately have predictive value. The “standard model” has stood unchallenged for many decades, and it is undoubtedly time that it be reviewed and refined.

References

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Eugenia Flores-Figueroa and Hector Mayani