Hematopoietic potential of neural stem cells

To the editor—In the February 2002 issue of *Nature Medicine*, Morshead *et al*.[1] report the inability to generate hematopoietic progeny from neural stem cells (NSCs), raising several issues that must be addressed to avoid misinterpretation. Specifically, although they were purported to be identical, a number of substantial deviations exist between this study and our experiments showing the neurohematopoietic potential of NSCs (ref. 2). The functional characteristics of the NSC cultures described by Morshead *et al*. show that the cells that they used are unlike any known NSC, particularly those used in our transdifferentiation experiments[2]. These authors' cultures become consistently transformed, a property that we and others have never observed. Neither human nor mouse NSCs undergo transformation with passaging, but rather exhibit growth factor dependency, unaltered growth kinetics and prompt differentiation upon growth factor withdrawal over time[3–6]. Furthermore, Morshead *et al*. report that "less than 1% of the cells composing an individual neurosphere are NSCs," which is far below the current standard in this system: from 8% (postnatal) to over 20% (embryonic)[3–6]. Thus, Morshead *et al*. injected at least 20 times fewer NSCs than in our study, using populations of NSCs that displayed altered growth and differentiation capacity. Moreover, as noted by these authors, "transformed, aggressively growing cells would progressively eliminate non-transformed cells," further exacerbating their NSC deficiency. Eventually, the combination of low NSC number and significant transformation found in the Morshead *et al*. cultures would lead to the transplantation of a negligible number of NSCs. These authors' use of a high sensitivity method to detect hematopoietic engraftment cannot possibly compensate for such severe deficiency, particularly since the kinetic parameters that apply to engraftment in standard repopulation experiments with blood stem cells cannot be extrapolated to the different phenomenon that is the object of these studies, namely the expression of an hematopoietic fate by NSCs.

So far, neuro–hematopoietic conversion has been reported by three independent groups[2,7,8]. In particular, Shih *et al*. confirmed the work by Bjornson *et al*. by using human NSCs (ref. 8). Although Morshead *et al*. suggest these results were due to hematopoietic contamination, closer examination of the methods employed by Shih *et al*. rules out this possibility. Furthermore, additional studies have reported that NSCs transdifferentiate into non-hematopoietic mesodermal derivatives[9]. Thus, the ability of NSCs to give rise to non- neural cells should be less of a question, particularly in light of the variety of freshly-isolated and cultured somatic stem-cell types which appear capable of transdifferentiation[10].

We suggest that a more parsimonious conclusion should be drawn from the study of Morshead *et al*., and submit that transdifferentiation experiments using transformed/transforming cultures with a negligible content of NSCs would necessarily yield an experimental outcome different than one using normal, highly clonogenic NSC cultures. The failure to detect transdifferentiation even at the single cell level is therefore not surprising. While we concur that hematopoietic transdifferentiation may represent a rare property of NSCs, we suggest that owing to disparate culture conditions and an unexplained NSC deficiency in the Morshead *et al*. experiments, it is inappropriate to compare this study to that of Bjornson *et al*.

ANGELO L. VESCOVI[1],
ROD RIETZE[2]
MARIA CRISTINA MAGLI[3], &
CHRISTOPHER BJORNSON[4]
[1]*Stem Cell Research Institute, DIBIT HSR, Milan, Italy*
[2]*The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia*
[3]*Consiglio Nazionale delle Ricerche, Pisa, Italy*
[4]*University of Washington, Seattle, Washington, USA*
Email: vescovi.angelo@hsr.it

To the editor—Three independent groups have previously reported that NSCs are capable of differentiating into hematopoietic cells[2,7,8]. In disagreement with these previous studies, Morshead *et al*.[1] report that hematopoietic competence is not a propensity of cultured NSCs, but a rare property of NSCs that may depend on genetic and epigenetic alterations. Is there a possible explanation to reconciling the differences between the three previous reports and the report by Morshead *et al*.? Using the SCID-hu mouse model, we have reported that cultured human NSCs possess *in vivo* hematopoietic potential[8]. Although neurospheres from bulk cultures were used, extensive analyses, including a stromal-coculture system[11,12] that can detect the presence of a single hematopoietic cell, were performed to rule out the possibility of contaminating hematopoietic cells in our NSC cultures. Our results demonstrate that cultured human neurosphere cells commit and differentiate into hematopoietic stem cells (HSCs) in intact human bone marrow in SCID-hu mice. We estimate that one in a hundred cultured human NSCs are capable of differentiating into HSCs, which are responsible for initiating hematopoietic reconstitution in secondary recipients. Several hundred individual neurospheres have been analyzed in our laboratory for their potential to differentiate into neural progeny *in vitro*, and we have never observed a single individual neurosphere that has lost its ability to generate neural progeny *in vitro*. These results suggest that cultured human NSCs that possess hematopoietic potential *in vivo* have also maintained their NSCs ability to generate neural progeny *in vitro*, and that they represent a totipotent neurohematopoietic stem-cell population in human brain tissues.

As the frequency of NSCs in human brain tissues is about 0.5–1%, we estimate that the frequency for the totipotent neurohematopoietic stem-cell population in human brain tissues is about 1 in 1–2 × 10^4. Our data correlate well with the 1982 study of Bartlett *et al*.[7] that showed neurohematopoietic stem-cell population in adult mouse brain at the same frequency. In a recent analysis of muscle differentiation potential of NSCs by Galli *et al*.[9], all of the clones tested were myogenic and one of the clones, 2H1, was the same used earlier by Bjornson *et al*. in the hematopoietic study[2], showing a multipotential for neural, hematopoietic and myogenic potentials. Analyzing blastocyst chimeras in chick and mouse embryos generated using cultured neurospheres, Clarke *et al*.[13] have reported that NSC-derived cells were reproducibly found in various organs of the embryo derived from all three germ layers, with the exception of