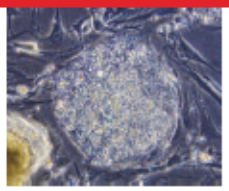

STEM CELLS IN FOCUS
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Stem-cell tagging shows flaws

Biologists have overlooked problems with a decades-old technique for marking cells, warn US researchers. They say their findings call into question some studies of adult stem cells. The dispute is part of an ongoing and heated debate within the stem-cell community about whether adult stem cells extracted from one tissue, such as bone marrow, can spawn the cell types of a different tissue, and so be used to repair damage caused by disease.

Cell biologists have long relied on a technique called thymidine labelling, whereby a group of chemicals such as bromodeoxyuridine (BrdU) are incorporated into the DNA of dividing cells.

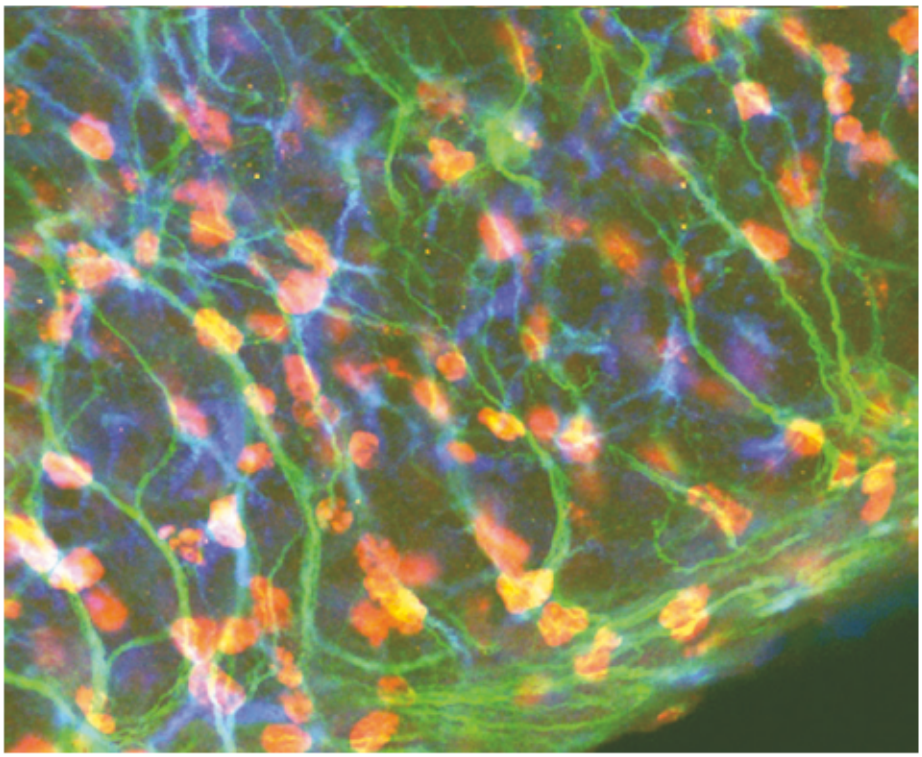
In some studies, BrdU is used to mark cells splitting at a particular time, allowing researchers to later examine where those cells migrated and what new cell types they generated. Often, stem cells are extracted from one tissue, marked with BrdU and then transplanted into a second tissue, such as the brain.

Now a team led by Catherine Verfaillie at the University of Minnesota, Minneapolis, is raising a red flag over the method. The group found evidence suggesting that many thymidine-labelled stem cells die after being transplanted into the brains of mice, and then release the chemical, which is taken up by neighbouring cells that are also dividing. This means that cells normally resident in the tissue could become marked and mistaken for transplanted cells.

The results were first published online in December 2005 (T. C. Burns *et al. Stem Cells* doi:10.1634/stemcells.2005-0463; 2005). "Hopefully, this will serve as a long-overdue wake-up call that will illustrate how misleading thymidine labelling can be," says Verfaillie.

Certain investigators who have used the method for many years say they already run rigorous control experiments that should avoid potential problems. One method is to transplant dead cells labelled with BrdU and compare the results to those obtained with live cells. Many researchers also use additional techniques to label their transplanted cells, such as green fluorescent protein (GFP), which is thought to be a reliable cell marker.

Even so, some experts say thymidine-labelling is so commonplace that biologists may be blasé about potential pitfalls and skimp on the controls. "It's important to have a caution about these issues," says neurobiologist Fred Gage at the Salk Institute for Biological Studies in San Diego, California.



T. BURNS

Chemicals used to mark stem cells to track their behaviour may be taken up by cells not originally tagged.

> "This will raise the ire of a lot of people."

Verfaillie says that the problems with BrdU could potentially affect any type of cell. But her study has focused on adult bone-marrow stem cells. Several researchers have reported that certain stem cells extracted from adult bone marrow and transplanted into the brains of mice can transform into new neurons or their supporting glial cells — fuelling the idea that these cells could repair brain disease or damage. Verfaillie says some of these results might be experimental artefacts, and that researchers should double-check their results: "In our hands it's all false positives," she says.

Cause to question

A study by Donald Phinney at Tulane University, New Orleans, on the properties of bone-marrow stem cells (G. C. Kopen, D. J. Prockop and D. G. Phinney *Proc. Natl Acad. Sci.* 96, 10711–10716; 1999), is among those questioned in Verfaillie's paper. Phinney counters that since Verfaillie's study, many groups, using different techniques, have independently reported that transplanted bone-marrow stem cells acquire properties of neurons and glial cells, and questions whether the phenomenon Verfaillie's team saw could be unique to their experiments. "I think it'll be controversial and raise the ire of a lot of people," he says.

There are additional, overlooked hazards of using BrdU, warns neuroscientist Pasko Rakic of Yale University in New Haven, Connecticut. One study from his lab showed that an injury can trigger neurons in the brain to start synthesizing new DNA, and that these cells can also take up the marker even though they are not actually dividing (C.-Y. Kuan *et al. J. Neurosci.* 24, 10763–10772; 2004). A second study from another lab also suggested that BrdU could boost the ability of bone-marrow stem cells to generate new cell types. This implies that some of the properties previously attributed to the cells may actually have been triggered by the chemical altering gene activity (T. Y. Qu *et al. Rest. Neurol. Neurosci.* 22, 459–468; 2004).

Such findings highlight the need for a broad rethink about how best to label transplanted cells, says Kenneth Chien, who studies heart stem cells at the University of California, San Diego. Biologists need "a more rigorous tool kit", he says — a repertoire of cellular markers that can unequivocally distinguish which cells have been transplanted, whether they are alive and precisely what they become. ■

Helen Pearson