Journal Club

# Adult stem cells: an alternative to embryonic stem cells?

The issue of research involving stem cells derived from human embryos is increasingly the subject of international debate. Scientists believe that research using embryonic stem cells offers great promise that could help improve the lives of those who suffer from diseases such as diabetes or Alzheimer. But this research raises profound ethical questions because extracting the stem cell destroys the embryo.

That is why US president George Bush announced August 2001 that he would allow the federal government to fund work on embryonic stem cells but only those cell lines derived before his announcement. Also, the practice of obtaining embryonic stem cells after his announcement will be punished. That reduces the number of cell lines to 64, as the National Institutes of Health (NIH) publicly posted in August.

Scientists suspect that, because many of these cell lines are in early stages, far fewer will prove to be of research quality. Also, they think that the NIH has established a low threshold of acceptability and that they will have to provide more information about them, such as evidence of pluripotency.

These are some of the reasons of why many scientists have redirected their research to use other sources, such as adult cells or umbilical cords. However, do adult stem cells have the same potential and offer the same benefits as embryonic stem cells? Adult stem cells have the potential to generate not only new stem cells but also several types of mature cells; neural stem cells, for example, can produce neurons and their supporting cells, glia. The results of a recent study show how plasticity of these adult stem cells could raise the possibility of using brain stem cells in the treatment of, for instance, neurodegenerative disorders such as Parkinson's disease.

A team led by Vaclav Ourednik and Jitka Ourednik of Harvard University has studied how many central nervous systems regions act as a reservoir of neural stem cells for subsequent use in homeostasis and repair[1]. They injected human neural stem cells into the developing brains of monkey fetuses, and the cells distributed themselves and appeared to segregate into two subpopulations: some contributed to corticogenesis by differentiating into neurons and glia, whereas others remained undifferentiated in a secondary germinal zone, or interspersed in brain parenchyma. These cells might provide a local resident pool for self-repair and plasticity; they could represent the stem-like cells extracted by several investigators and might be useful in the treatment of degenerative neurological disorders.

The research team was unable to measure exactly how many of the injected cells survived, but confirmed that a 'large number' migrated through the large expanse of the primate cerebrum, which suggests that migration might be a fundamental stem cell property limited only by the available terrain. In rodents, neural stem cells have been well studied for transplant-based approaches to gene therapy and/or cell replacement in diseases characterized by extensive or global abnormalities. The results of this study suggest that this approach might be feasible in large primates and possibly humans.

1 Ourednik, V. *et al.* (2001) Segregation of human neural stem cells in the developing primate forebrain. *Science* 293, 1820–1824

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# New molecular beacons for targeting double-stranded DNA under native conditions

The sensitive and specific detection of given nucleic acid sequences within complex biological mixtures is an essential aspect of many areas of research and diagnosis. Since they were first described in 1996 (Ref. 1), molecular beacons (MBs) have represented one of the most promising new techniques to accomplish this task. An MB consists of an oligonucleotide complementary to the sequence of a target DNA or RNA molecule, flanked by short 5¢ and 3¢ extensions, the ends of which carry a fluorescent and a quencher group, respectively. Because the extension sequences are complementary to each other, they hybridize to generate a stem-loop conformation. This brings the fluorophore and the quencher in close proximity, so that the fluorescence of the former is dissipated as heat by the latter instead of being emitted as light. However, if the MB encounters a target nucleic acid, a hybrid is formed that is more stable than the hairpin structure of the isolated probe. As a result, the beacon undergoes a spontaneous conformational change that separates the fluorophore from the quencher, thus restoring a fluorescence signal proportional to the quantity of the target.

Unlike alternative detection techniques, MBs do not require probe–target complexes to be immobilized on solid supports or to be separated from unhybridized probes (which do not fluoresce). They have therefore been extremely useful for monitoring the amplification of nucleic acids in real-time, as well as for detecting specific RNAs in living cells. More recently, the introduction of fluorophores emitting lights of different wavelengths has been coupled to the single base pair mismatch discrimination ability of an appropriately designed MB to perform a so-called spectral genotyping of human alleles.

In spite of these exciting applications, a serious limitation of this class of probes was the need for the target nucleic acids to be denatured in order to bind to the beacons. Kuhn *et al.*[2] now describe how to overcome this problem by using peptide nucleic-acid (PNA)-based MBs to directly target double-stranded DNA. In the first step of the new assay, two pyrimidine *bis*-PNA openers hybridize to closely located short oligopurine sequences

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within the target DNA duplex (such a site is predicted to statistically occur once in every 400–500 bases of random DNA). This generates a local displacement of the opposite strand of the target, which can in turn hybridize to a complementary PNA beacon to generate a quaternary complex, named a PP-loop. The authors show that the formation of this structure, which can be detected by gel-shift assay and only occurs in the presence of a specific target, is associated with a rapid increase in fluorescence displaying signal-to-background ratios of around ten.

The two PNA beacons (designated MB1 and MB2) described in this study shared the same recognition sequence and presumably both formed a closed loop in the absence of the target, although they were not designed to adopt the standard stem-loop structure. However, they differed in the arrangement of the fluorophore and quencher groups, as well as in net electrostatic charge. Further studies will be needed to clarify whether these differences are responsible for the diverse behavior of the two probes, only one of which (PNA MB2) was able to hybridize to the pre-opened target at room temperature. Finally, the authors provide evidence that, unlike that of classical DNA beacons, the fluorescence of PNA MB2 is not affected by the presence of *Escherichia coli* single-stranded DNA-binding protein. Considering that PNA is not a substrate for enzymes that process DNA and RNA, this is an important observation as it suggests that PNA MB2 could be directly applied to the analysis of non-deproteinized samples.

Although only future tests with progressively 'dirtier' analytes will eventually tell to which extent this technology will be applicable, the features of the new PNA beacons reported by Kuhn *et al.* are undoubtedly a major step ahead towards the highly sensitive probing of native samples, both for diagnostic and for research purposes.

1 Tyagi, S. and Kramer, F.R. (1996) Molecular beacons: probes that fluorescence upon hybridization. *Nat. Biotechnol.* 14, 303–308
2 Kuhn, H. *et al.* (2001) PNA beacons for duplex DNA. *Antisense Nucleic Acid Drug Dev.* 11, 265–270

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In Brief

## UK funds spin-out research

The UK government is to boost the entrepreneurial spirit in UK universities, colleges and hospitals by giving £120m, to help researchers commercialise the results of their work (*Chemistry and Industry*, 15 October). The bulk of the funds (£80m) will help institutions strengthen their links with businesses and the community. A further £15m will be 'seed' funding to advance research to the point of commercialisation. Funding will also be used to teach entrepreneurial skills to science and engineering students. *MJD*

## Terpenes boost antibiotic action

Naturally occurring sesquiterpenoids produced by many plants often act against insect infestation. New research at the Food Research Institute of the University of Wisconsin (Madison, WI, USA) has shown that sesquiterpenoids such as nerolidol, bisabolol, farnesol and apritone can also enhance the activity of antibacterial agents (*Chemweb.com*, 1 October). Eric Johnson and coworkers found that these sesquiterpenoids, which are lipophilic, disrupt bacterial cell membranes, making then permeable and easier for antibiotics to enter and kill the cells. The effect appears to be non-specific but is more pronounced in Gram-negative bacteria. *MJD*

## Bacteria shun the limelight

Scientists at Clark University (Worcester, MA, USA) have found that bacterial colonies irradiated with UV light will migrate to form large ring-shaped patterns thus indicating that stress caused by UV exposure is having an effect on the bacteria's metabolism (*Physics Review Letters*, 8 October). Cultures of the soil bacterium *Bacillus subtilisin* grown on a nutrient-rich medium were exposed to a uniform UV light, and began to migrate away from the centre of the original colony. When the light was turned off, the bacteria repopulated the areas that had been vacated. The Clark team propose that UV-induced stress causes the bacteria to become sensitised to waste metabolites that accumulate at the colony centre, and that cells at the colony's edge emit chemicals to attract the others to regions of uncontaminated media. These results could have implications for understanding the effects of UV exposure on biological systems due to depletion of the ozone layer. *MJD*

## Nanomaps are a one-way street

Japanese nanotechnologists have prepared microlithographic tracks of the transport protein kinesin that can ferry microtubules in a single direction (*Biophysical Journal*, September issue). The team from the National Institute of Advanced Industrial Science and Technology (Ibaraki, Japan) etched nanoscopic routes, complete with direction 'arrows', in a glass surface. These were coated with kinesin, and then a solution of microtubules was added, which 'walked' along the kinesin tracks. The etched arrows served to redirect 'lost' microtubules back to the main route by circulating them in one direction before a kinesin molecule retrieved them. *MJD*

## 11 September fallout

Not surprisingly, considering the scientific community's heavy reliance on travel and the free exchange of data, science hasn't escaped the aftermath of the terrorist attacks in the USA on 11 September. For example, The American Society for Microbiology, which had scheduled its conference in Chicago on 22–25 September, decided to postpone the meeting until December. There could also be some restrictions on data access: 'At present, we distribute and share scientific information without regard to where it's going' says Graham Cameron, joint head of the European Bioinformatics Institute (Hinxton, UK). He speculates that the US government and its allies could demand that certain countries are excluded from access to a range of scientific data such as the genome sequences of pathogenic microorganisms. Interestingly, at the time of writing (22 October), there appears to be no problems accessing most pathogen genome sequences – at least from

 