MedicalXpress

Cloned mice created from fully differentiated cells, a milestone in cloning research

October 2 2006

New research dismisses the notion that adult stem cells are necessary for successful animal cloning, proving instead that cells that have completely evolved to a specific type not only can be used for cloning purposes, but they may be a better and more efficient starting point. As proof, researchers report they created two mouse pups from a type of blood cell that itself is incapable of dividing to produce a second generation of its own kind.

This is the first demonstration that an animal can be derived directly from a fully differentiated cell, report lead researchers Xiangzhong (Jerry) Yang, Ph.D., of the University of Connecticut, and Tao Cheng, M.D., of the University of Pittsburgh, in the journal *Nature Genetics*.

Moreover, they say results of their studies provide compelling evidence that Dolly the sheep and other mammals cloned by somatic cell nuclear transfer were most likely derived from fully differentiated cells, not adult stem cells, as most have argued in the nine years since Dolly was first created. Because stem cells have the ability to self-renew and differentiate into any specialized cell type, they have been heralded for their promise for treating a variety of diseases and conditions. Yet, even for cloning of an embryo to the blastocyst stage, from which embryonic stem cells can be generated, adult stem cells have yielded disappointing results, with success rates in the range of 1 to 5 percent.

Somatic cell nuclear transfer (SCNT), the scientific term for cloning, involves creating an embryo by using a nucleus that's been removed from

a somatic cell – any cell other than a reproductive cell – and transferring it into an unfertilized egg that has had its chromosomes removed. Because the resulting new embryo contains the entire genome of the donor somatic cell it is an identical copy. This cloned embryo is then implanted into a surrogate mother, and, if the process is successful, is carried to term.

In their studies, the researchers compared the efficiency for cloning mice using a fully differentiated blood cell called a granulocyte with its ancestor cells at different stages: hematopoietic stem cells, which are found in bone marrow and give rise to all red and white blood cells, and progenitor cells. Granulocytes are well characterized white blood cells unique for their segmented nuclei and the numerous granules in the cells' cytoplasm.

Surprisingly, the granulocytes were the most efficient donor cells for nuclear transfer among the different lineage cells, with 35 to 39 percent becoming a blastocyst, an early embryo consisting of about 100 to 150 cells, compared to 11 percent for the progenitor cells and only 4 percent for the stem cells. Only the granulocytes were able to produce two live cloned pups, although both died within a few hours of birth. As a control, the researchers performed nuclear transfer using embryonic stem cells; 49 percent developed to the blastocyst stage and 18 cloned pups were born.

"Our results clearly demonstrate that there is no apparent advantage in using either adult stem cells or progenitor cells over fully differentiated cells as nuclear donors. To the contrary, we found that cloned pups can be produced from adult, fully differentiated somatic cells, a conclusion that goes against popular opinion and current hypotheses," says Dr. Yang, animal science professor, director of the University of Connecticut's Center for Regenerative Biology and co-corresponding author of the study.

"Even we were surprised to find fully differentiated cells were more efficient for cloning, because granulocytes are not capable of dividing. In fact, we repeated our experiments six times just to be sure. Now we can say with near certainty that a fully differentiated cell such as a granulocyte retains the genetic capacity for becoming like a seed that can give rise to all cell types necessary for the development of an entire organism," adds co-corresponding author Dr. Cheng, associate professor of radiation oncology at the University of Pittsburgh School of Medicine and director of stem cell biology and co-leader of the cancer stem cell program at the University of Pittsburgh Cancer Institute.

Previous attempts by scientists to produce animal clones directly from fully differentiated B cells, T cells and neurons had failed beyond the blastocyst stage. Only with a second step that involved combining the blastocyst with a fertilized embryo, which produces what biologists call a chimera, or by performing another nuclear transfer using the embryonic stem cells derived from these blastocysts, could "cloned" pups be produced. Even so, other researchers have countered these are not bona fide clones because they possess chromosomes that are not identical to those of the original donor nucleus.

Since Dolly, animal cloning using adult cells has been accomplished in more than a dozen mammalian species, but the process is highly inefficient. Even if the reconstructed eggs survive to the blastocyst stage, only a handful, at most, of these result in live young when implanted into a female.

Many have attributed cloning's limited success to a theory that clones must be derived from adult stem cells, which reside in a specific area of each tissue and remain quiescent until they are activated by the presence of disease or tissue injury. Yet, if this were true, Drs. Yang and Cheng point out, the results of their studies would have found the adult stem cells to be more efficient than the other, more differentiated cells.

"Of the 1,828 nuclear transfers we performed with stem cells, very few could develop to the blastocyst stage and not one clone was produced. With such odds, it's hard to believe that Dolly and other cloned animals could have possibly been derived from adult stem cells. Much more likely is that these animals were derived from fully differentiated tissue cells," Dr. Yang argues.

While more research is needed to determine if what they found with hematopoietic cells will be true for cells of other tissue types, the investigators say their current studies may have important implications for regenerative medicine, since the findings suggest the potential of adult stem cells in this arena may be more limited than previously thought. However, of particular interest to Dr. Cheng is the relevance of their findings to cancer stem cell research.

"An interesting question to me is whether SCNT can play a role in understanding or even reprogramming the behavior of cancer stem cells. Such studies may potentially reveal a new set of molecular targets that could aid in the treatment of cancer," says Dr. Cheng.

Source: University of Pittsburgh