# Scientists report new strategy to create genetically-modified animals

September 20 2007

Researchers at the University of Pennsylvania School of Veterinary Medicine have demonstrated the potential of a new strategy for genetic modification of large animals. The method employs a harmless gene therapy virus that transfers a genetic modification to male reproductive cells, which is then passed naturally on to offspring.

Ina Dobrinski, associate professor and director of the Center for Animal Transgenesis and Germ Cell Research at Penn Vet, and her colleagues introduced adeno-associated virus, AAV, to male germline stem cells in both goats and mice. The study showed that AAV stably transduced male germ line stem cells and led to transgene transmission through the male germ line.

The findings, available online in The FASEB Journal and in the February 2008 print edition, are the first report of transgenesis via germ cell transplantation in a non-rodent species, a promising approach to germ line genetic modification. It also demonstrates that germline transduction and germ cell transplantation in large animals provides an approach that is potentially less costly than microinjection and cloning, the traditional methods used to generate transgenic large animal models for biomedical research.

Researchers used mouse germ cells harvested from experimentally induced cryptorchid donor testes that were then exposed in vitro to AAV vectors carrying a green fluorescent protein transgene and transplanted to germ cell-depleted recipient testes, resulting in colonization of the

recipient testes by transgenic donor cells.

When researchers mated these recipient males with wild-type females, 10 percent of offspring carried the gene originally introduced into the transplanted germ cells, meaning the genetic modification had been passed on. To broaden the approach to non-rodent species, AAV-transduced germ cells from goats were transplanted to recipient males in which endogenous germ cells had been depleted by fractionated testicular irradiation. Transgenic germ cells colonized recipient testes and produced transgenic sperm. When semen was used for in vitro fertilization, 10 percent of embryos were transgenic.

“Initially, the team used the established germ cell transplantation model in the mouse to investigate whether AAV-mediated transduction of germ cells was possible and could result in transgene transmission,” Dobrinski said. “To broaden the applicability of the results for different mammalian species, the approach was then applied to a large animal species in which germ cell transplantation-mediated transgenesis would provide an important alternate approach to the generation of transgenic animal models for biomedical research.”

Currently, somatic cell nuclear transfer or pronuclear injection is used to generate transgenic animals. These inefficient and difficult methods also carry a risk of producing offspring with developmental abnormalities. The use of retroviral or lentiviral vectors has been reported in rodents, but it requires that animals be handled and maintained under higher biosafety precautions that render this approach less practical for transgenesis in large animal species. In contrast, animals exposed to AAV can be maintained under standard husbandry conditions.

AAV is a dependent virus that carries no disease and causes only a very mild response from the immune system. Because AAV can infect both dividing and non-dividing cells and passes its genome, it is considered an

excellent candidate for use in gene therapy.

Source: University of Pennsylvania