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LETTER TO EDITOR

The contribution of large animal models for studies into developmental plasticity and regenerative sciences

Prof. Dr. med. vet. Heiner Niemann*

Since the report of "Dolly", the cloned sheep, great progress has been made in the improvement of cloning technology and viable offspring have been cloned in several mammalian species, including sheep, cattle, pig, goat, horse, mule, cat, mouse, rabbit, rat, dog, wolf, ferret, buffalo, camel. Somatic cell nuclear transfer involves five major steps, i.e. enucleation of the recipient oocyte, transfer of the donor cell, fusion, activation and culture of the reconstructed embryo. Cloning technology is still characterized by relatively low success rates and a certain proportion of the offspring shows abnormalities (specifically ruminants, mice) known as the "Large Offspring-Syndrome" (LOS). In cattle, success rates are higher (15-25% live offspring based on the number of transferred embryos) and viable offspring can be produced from cloned embryos on a regular scale. Due to important modifications of the cloning protocol, we can now routinely produce cloned transgenic pigs with high levels of efficiency. Transfer of nuclear transfer complexes into oviducts of recipient animals prior to ovulation significantly improved success rates of porcine cloning. Fourteen pre-ovulatory transfers resulted in 11 pregnancies with a total of 56 cloned transgenic piglets born. All offspring had normal birth weights (1.0-1.5 kg) and showed no malformations. Average litter size was 5.2; the overall efficiency was 3.1% and 4.4% when related to pregnant recipients.

The underlying mechanism for the correct epigenetic reprogramming of the somatic donor cell with the induction and sustaining of a regular preimplantation and fetal development of the reconstructed embryo are poorly understood. DNA methylation plays an important role in the epigenetic regulation of selective transcription in early mammalian development. Following fertilization, the preimplantation embryo undergoes species-specific waves of de- and re- methylation. These processes appear to be sensitive to the environmental disturbances associated with assisted reproduction technologies (ART) since cattle derived by in vitro methods frequently exhibit condition known as "large offspring syndrome" and children produced by ART exhibit a slightly increased frequency of Beckwith-Wiedemann Syndrome, a rare epigenetic disease. To understand ART induced epigenetic alterations, we screened a panel of 42 amplicons representing 25 developmentally important genes on 15 different bovine chromosomes (a total of 1069 CpG sites) and, in two rounds of selection, arrived at a subset 22 informative amplicons (hot spots) covering 450 CpG sites in 19 genes. The "barcode" pattern from these amplicons demonstrates the DNA-methylation differences between somatic cells, in vivo developing embryos, embryos produced by in vitro fertilization (IVP), and embryos produced by somatic cell nuclear transfer (SCNT). As expected, somatic cells such as (peripheral blood mononuclear cells (PBMCs) and fibroblasts (two SCNT donor cell lines) showed higher levels of CpG methylation than embryonic samples and extensive demethylation of fibroblast DNA could be seen following fusion with enucleated oocytes. In a comparison of the three classes of embryos, the in vivo embryos generally showed more CpG methylation in the informative amplicons than their IVP and SCNT counterparts. Our results revealed several informative sites where DNA methylation is correlated with diagnosis or quality control of mammalian preimplantation embryos and for studying the reprogramming of nuclear transfer derived stem cells.

Somatic nuclear transfer holds great promise for significant improvements in the production of transgenic livestock. Donor cells can be successfully transfected with different types of gene constructs and viable cloned transgenic offspring with stable integration have been obtained in sheep, cattle, goats, and pigs. The main advantage is the possibility of selecting the

Institute of Farm Animal Genetics (FLI)
Mariensee, 31535 Neustadt, Germany

Tel.: 0049-(0)5034-871-136; Fax: 0049-(0)5034- 871-101

*heiner.niemann@fli.bund.de

donor cells for optimal integration and expression of the transgenic construct and their direct use in nuclear transfer as well as the possibility of targeted genetic modifications. Most groups interested in large transgenic animals have therefore switched from microinjection to nuclear transfer for the production of transgenic livestock.

The first area of application is in biomedicine, especially gene pharming and xenotransplantation. Our own research is related to the production of multi-transgenic pigs from which organs show improved survival in a xenotransplantation setting. Porcine xenografts are considered the method of choice for closing the growing gap between terminally ill patients and the availability of human allotransplants. Transplantation of porcine organs to humans requires genetic modification of the porcine genome to reduce or eliminate immunogenicity. The hyperacute rejection response (HAR) which was the premier hurdle in porcine–to-human xenotransplantation, can already be overcome in a clinically relevant manner by expression of human complement regulatory proteins in transgenic pigs and/or by knockout of the critical galactosidase epitopes (1,3 α-gal) in the porcine genome. However, despite severe immunosuppressive treatment the acute vascular rejection (AVR) with the disseminated intravascular coagulation (DIC) as the preeminent feature and the cellular rejection remain major obstacles for long term survival of a porcine xenograft. DIC is frequently observed in a pig-to-primate xenotransplant model and is caused by activation of the endothelial cells mainly attributed to incompatibilities between human and porcine coagulation factors. The goal the research in our laboratory is the production and characterization of improved lines of multi-transgenic pigs targeting AVR and specifically this coagulation disorder. We have produced and characterized transgenic pigs expressing constructs for the human complement regulators CD 55, CD 59, thrombomodulin (hTM), human A20 gene (hA20) and human hemoxygenase-1 (hHO-1). HHO-1 has primarily anti-apoptotic and cell protective properties. The hA20 molecule possesses protective features against inflammatory and apoptotic stimuli in endothelial cells. Thus transgenic expression of these genes in pigs may be promising to prolong survival of porcine xenografts.

Nuclear transfer can also serve as an important tool in basic biological research. Important areas in biological research are related to differentiation, pluripotency, reprogramming, epigenetics, telomere biology, cancer, ageing, etc.. A recent example from our research is the discovery of a genetic program at morula-blastocyst transition which sets telomere length for life in mammalian species. For human medicine, the potential of creating patient specific stem cells to derive therapeutically useful cells thereof, would be of utmost importance. With the advent of somatic nuclear transfer, biology has been revolutionized and a number of longstanding dogmas in science have to be reconsidered. This relates in particular to the question of toti-or pluripotency that now are to be evaluated as the status of an individual DNA and is not permanently related to a certain cell type. This unexpected enormous molecular and cellular plasticity paves the way to novel therapies.

Further refinements of this technology will allow numerous novel application models and will also enhance productivity and diversification in animal production.

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