

Production of Cloned Piglets from Human CD59-Transgenic Embryonic Germ Cells for Reduction of Hyperacute Rejection in Pig-to-Human Xenotransplantation

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Production of transgenic pigs for human complement regulatory protein CD59 may reduce hyperacute rejection (HAR), the first and most critical immunological hurdle in the development of xenogeneic organs for human transplantation. The aim of this study is to produce CD59-transgenic pigs using nuclear transfer (NT) of embryonic germ (EG) cells, undifferentiated stem cells derived from primordial germ cells. Since EG cells can be cultured indefinitely in an undifferentiated state, they may be more suitable for nuclear donor cells than somatic cells which are often unstable and have limited lifespan in primary culture. Use of EG cells would be particularly advantageous to provide inexhaustible source of transgenic cells for NT. Human CD59 gene under the regulation of cytomegalovirus promoter was introduced into porcine EG cells, and transgenesis of EG cells was confirmed by PCR. These cells were cultured in feeder-free state and reacted with human serum containing xenoreactive natural antibody and complement. Human serum did not affect the survival of CD59-transgenic EG cells, whereas approximately 60% of non-transgenic control EG cells were died from complement-mediated cytotoxicity. Oocytes reconstructed by NT of CD59-transgenic EG cells developed to blastocysts with the rate of 12.3% (31/253). Analyses of individual blastocysts by PCR indicated that 80.6% (25/31) of embryos contained CD59 transgene. Transfer of 160 NT embryos at their 1- to 2-cell stage into the oviduct of one recipient

resulted in the birth of five piglets. Among these offspring, one was stillborn, two died within 24 h after birth, and two are currently alive with no overt health problem. PCR analyses of DNA from the skin biopsies confirmed that all five piglets were transgenic for CD59. These results demonstrate that human CD59–transgenic pigs could effectively be produced from EG cell NT, and such transgenic pigs may be used for organ donors in pig–to–human xenotransplantation.

Key words) *Embryonic germ cell, Transgenic pig, CD59, Nuclear transfer, Xenotransplantation*