구두발표-6

Dogs Cloned from Adult Cells by Nuclear Transfer

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Several mammals,including sheep, mice, cattle, goats, pigs, rabbits, cats1, a mule2, a horse3 and a pair of rats4 have been cloned by somatic cell nuclear transfer into enucleated oocytes [SCNT]; however, SCNT in dogs has been hampered by limited success in maturing canine oocytes in vitro. Here we report the successful cloning of two Afghan puppies by SCNT of adult fibroblasts into dog oocytes matured in vivo. Canine SCNT may prove invaluable for: endangered species preservation; therapeutic cloning (once embryonic stem cells are established in dogs); and, with the dog genome sequence now complete, for discovering the respective genetic and environmental contributions that account for the markedly diverse biological and behavioral traits of the many canine breeds.

Cloning success depends on the quality, availability and maturation of the unfertilized oocyte; unlike other mammals, dogs ovulate at first meiotic prophase, with oocytes maturing for 2-3 days in the oviduct's

distal regions. Westhusin et al6 successfully reconstructed intra- and interspecific embryos by canine SCNT into both bovine and canine oocytes, but without viable offspring. We obtained in vivo matured oocytes at metaphase II at ~72 hours after ovulation by flushing oviducts. Donor fibroblasts were obtained from an ear skin biopsy of a male Afghan hound (Fig. 1A) and cultured for two to five passages. For SCNT (see supplementary information), the chromosomes of the unfertilized oocytes were removed by micromanipulation,and a single donor cell was transferred into each enucleated oocyte. The couplets were fused,and only successfully fused couplets (75 %) were activated; the activated oocytes, or in vitro cultured embryos, were then transferred into oviducts or uterine tubes of recipient dogs, depending on each NT-construct's developmental stage. An average of 12 oocytes was collected from each female, and a total of 1,095 reconstructed embryos were transferred into 123 recipients.

Three pregnancieswere confirmed by ultrasonography at 22 days of gestation in recipients after transfer of NT-constructs. Pregnancy establishment was only confirmed after embryo transfers [ETs] at very early stage NT-constructs, i.e. < 4 hours after oocyte activation, suggesting the importance of early ETs for assisted reproductive technologies [ART] in dogs. One fetus miscarried and two others were carried to term. The first cloned dog, named "Snuppy", for Seoul National University puppy, resulting from embryo transfer into a yellow Labrador female surrogate (Fig 1A, B), was delivered by caesarian section at 60 days after embryo transfer (ET), with a birth weight of 530 g. The second cloned dog, carried by a mixed breed surrogate, also delivered at 60 days post-ET and weighed 550 g. It experienced neonatal respiratory distress during its first week, seemed to recover, but died on day 22 due to aspiration initial results do not indicate major structural anomalies, but detailed investigations are underway.

To confirm the genetic identity of the cloned dogs, microsatellite analysis was performed by genomic DNA testing of the donor dog (from blood leukocytes and cultured nuclear donor fibroblasts), the cloned dogs (from tail tissue fragments), and surrogate females (from blood leucocytes). Analysis of 8 canine-specific microsatellite loci confirmed that the cloned dogs were genetically identical to their donor dog (supplement Table 1). The efficiency of cloning in the present study is

still very low (2 dogs from 123 recipients) compared to the rate for cats1 and horses3. Clearly, cloning in dogs is inefficient with fetal and newborn losses. However, this work adds the dog to the list of mammals that have been successfully cloned from adult somatic cells.

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