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Mutation and Cloning Efficiency

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Opinion Piece

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SOMATIC CELL CLONING by nuclear transfer has been performed on a number of species, including sheep, mice, pigs, cow, cats, goat, rabbit, dogs, and horses (Xuemei et al., 2003). Yet, cloning efficiency, as determined by the number of offspring produced over the total number of oocytes injected, is fairly low (Hill et al., 2000; Wakayama, 2003). For example, Wakayama (2003) observed that cloning efficiency ranged from 1% to 2% in mice, and Wells et al. (1999) cloned calves from granulosa cells with a cloning efficiency of about 1.8%. In addition to cloning efficiency, the health and viability of clones is low as compared to those produced by normal reproduction (Cibelli et al., 2002), with clones often showing a high incidence of developmental abnormalities (Garry et al., 1996). For example, Hill et al. (1999) did a thorough analysis of cloned calves and fetuses, and observed cardiopulmonary and placental abnormalities, including neonatal respiratory distress and placental edema in almost all the clones. In addition, mouse clones have a shorter life span than uncloned mice, and Dolly, the famous cloned sheep, showed symptoms of arthritis often observed with aging (Ogonuki et al., 2002; Dyer, 2002).

A number of reasons have been suggested for the low cloning efficiencies and survival rates, and for the reduced viabilities of clones, including incomplete epigenetic reprogramming of the donor cell nuclei, histone acetylation and DNA methylation, cell-cycle status of donor cell, telomere length of cloned oocytes, and X-chromosome inactivation (Wilmut et al., 2002; Lanza et al., 2000). We would like to emphasize that another factor may also reduce cloning efficiency: the accumulation of new deleterious mutations over time in the somatic cells that are used for cloning.

HIGH RATE OF GENETIC DAMAGE IN SOMATIC CELLS

Recent studies show that rates of spontaneous deleterious gene mutations, trinucleotide repeats, chromosomal rearrangements, and aneuploidy in germ and somatic cells are higher than was thought in the past (Crow, 2000; Roland, 1999). For example, it has been estimated that there are at least three to six new recessive deleterious mutations in each human (Eyre-Walker and Keightley, 1999), and about one gamete in a thousand contains DNA transposition events that are often the cause of chromosomal rearrangements (Muotri et al., 2005). The *in vitro* and *in vivo* somatic cell rates of mutation are about three to 10 times higher than germinal rates (Neel, 1983; Drake et al., 1998). Thus, somatic cells may carry even more new deleterious mutations than germinal cells. The accumulation of deleterious mutations and the occurrence of aneuploidy in somatic cells during development not only cause cancer (Simpson, 1997; Hanks et al., 2004) but also reduce the fitness and lifespan of the hosts (Odagiri et al., 1999; Woodruff and Nikitin, 1995). Deleterious genetic damage may also reduce the health, fitness, and lifespan of clones that are formed from somatic cells.

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ROLE OF NEW GENETIC DAMAGE IN CLONING

The possible effect of new deleterious mutations in somatic cells on cloning efficiency and viability of clones has indirectly been tested by cloning from various somatic cell types from adults, newborns, and fetuses (Kato et al., 2000; Hill et al., 2000). In these studies, the cells from newborns showed higher cloning efficiency, a result that was also observed in the early cloning experiments with frogs where blastula cells of various stages were used for cloning (Briggs and King, 1952). Fetal loss and low viability were often observed in clones derived from adult cells. Bernstein et al. (1996) have also proposed that cells from ear and skin tissue are less suitable for cloning due to genetic damage by ultraviolet light, and Kato et al. (2000) have suggested that somatic mutations could lower cloning efficiency and be responsible for developmental abnormalities. Serial cloning experiments by somatic nuclear transfer show decreased efficiency and increased embryonic and fetal mortality with each generation (Kubota et al., 2004). This is expected, since clones made from clones would have more cell divisions and, hence, more new deleterious mutations.

One possible reason for the short life span of Dolly was aging effect (Roland, 1999; Shiels et al., 1999). She developed symptoms of arthritis and joint disease common in middle-aged sheep. The role of somatic mutations on aging has been discussed by many authors (Woodruff and Thompson, 2003). Thus, the accumulation of deleterious mutations in the udder cells from which Dolly was cloned could have been one of the reasons for her poor health. A low rate of homologous recombination in somatic cells is considered to be another obstacle to successful cloning (Cibelli et al., 2002). This could be indirectly related to the fact that deleterious mutations cannot be removed in bunches by recombination in somatic cells. Hence, clones from older cells would be less fit due to additional genetic damage from the accumulation of new deleterious mutations.

In support of the role of mutations in cloning, it has been observed that the survival rate of embryos is higher when embryonic stem (ES) cells are used for cloning (Rideout et al., 2000). This is due to reduced reprogramming requirement and low mutation rates of the ES cells. Stem cells may be less prone to the accumulation of mutations, since DNA strands with higher amounts of genetic damage are eliminated as proposed by the immortal strand hypothesis (Cairns, 1975). Use of ES cells may overcome some of the mutation problems, but it has been observed that ES cell lines also accumulate mutations over time, and should be used with caution and be monitored regularly for DNA and chromosomal changes (Anirban et al., 2005). Some organisms have evolved strategies to eliminate cells with damaged DNA before they enter mitosis, thereby reducing the accumulation of mutations (Raff et al., 2003).

CONCLUSION

Cloning has promising applications, but the major problems of low cloning efficiency, and poor fitness and viability of clones must be solved. We propose that the accumulation of new deleterious gene and chromosomal mutations in somatic cells could be one of the causes of reduced cloning efficiency and compromised health of cloned animals. The tactic of using younger donor cells could reduce some of these cloning problems. In some cases, this would require the cryopreservation of younger cells for use in future cloning experiments.

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