DEVELOPMENT

Faithful Expression of Imprinted Genes in Cloned Mice

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Phenotypic anomalies have been observed among animals cloned from somatic cells, putatively caused by epigenetic alterations, especially those of imprinted genes (*1*). However, the complexity of potentially contributory technical factors associated with nuclear transfer (NT) experiments could obscure any inherently biological ones. We therefore sought to isolate some of these complex factors by describing the patterns of development and gene expression of mice cloned by NT from fresh or primary cultured cells (cumulus, immature Sertoli, and fetal and adult fibroblast cells) under carefully standardized experimental conditions as described previously (*2*, *3*).

About 2.8% of morulae/blastocysts derived from such NT developed to term after transfer into recipient uteri. Most (144/155, 92.9%) of the resultant newborn pups were healthy and rapidly gained active movement. Because parent-specific monoallelic expression of imprinted genes is critically important for normal embryo development (*4*), we determined the allelic pattern of imprinted gene expression in Sertoli cell–derived NT F1 (C57BL/6 × JF1) fetuses and placentas at embryonic day 9.5 (E9.5). The choice of parental allele expressed {determined with allelic polymorphisms [see methods in supplementary material (*5*)]} was faithful to that previously described for fertilization-derived embryos (*6*) in all (n = 9) fetuses and placentas and for all imprinted genes investigated: *Igf2* and *Peg1/Mest* (paternally expressed) and *Igf2r*, *H19*, *Meg1/Grb10*, *Meg3/Gtl2*, and *p57^{Kip2}* (maternally expressed). Imprinting memory established during gametogenesis is thus stable and not readily transmuted within oocytes or embryos after NT.

We extended this analysis to a relative estimation of corresponding transcript levels in fetuses and placentas at midgestation (E12.5) by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) (*5*). Expression of two imprinted (*Meg1/Grb10* and *Peg1/Mest*) and two nonimprinted (*Igfbp2* and *Esx1*) genes was significantly reduced in the chorioallantoic placentas of four immature Sertoli cell–derived clones compared with controls (see Web fig. 1) (*5*). Contrastingly, the corresponding cloned fetuses exhibited steady state mRNA levels for these genes within the control range. Transcript levels for imprinted genes *Igf2* and *H19* [reportedly prone to cloning-associated anomalies (*7*)] rarely differed from those of controls in both cloned fetuses and placentas.

The placentas of cloned mice at term were consistently two to three times larger than those of controls (0.28 ± 0.01 g compared with 0.11 ± 0.01 g; $P < 0.00005$). Gene expression in term placentas of clones derived from either immature Sertoli cells or adult-derived cumulus cells exhibited a significant reduction in mRNAs for three imprinted and four nonimprinted genes compared with control placentas (Fig. 1). Contrastingly, the levels for *Igf2* and *H19* were within the control range. Relative transcript levels in clones and their associated placentas at E12.5 and term are therefore apparently superimposable. We confirmed by immunohistochemistry that for *Igfbp2*, *Igfbp6*, and *Meg1/Grb10* at least, decreased mRNA reflected a genuine reduction in average steady state mRNA levels within cells. The selective, nonstochastic reduction in mRNA levels we observed thus suggests that the genes responsible for the placental phenotype are regulated by a common upstream function independent of genomic imprinting and prone to dysregulation by NT cloning.

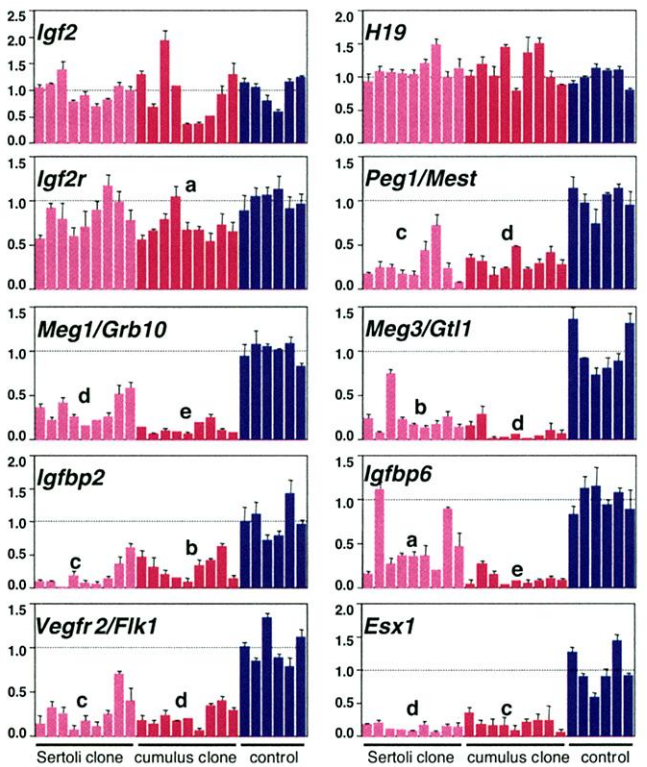

Fig. 1. Reduced transcript levels in clone-derived placentas at term. Quantitative PCR analysis (*5*) shows a significant reduction of mRNA levels of three imprinted genes (*Peg1/Mest*, *Meg1/Grb10*, and *Meg3/Gtl2*) and four nonimprinted genes (*Igfbp2*, *Igfbp6*, *Vegfr2/Flk1*, and *Esx1*) in placentas of both Sertoli cell– and cumulus cell–derived clones compared with controls (=1.0) with the same genotype (B6D2F1). Transcript levels for *Igf2* and *H19* are within the control range. a, $P < 5 \times 10^{-3}$; b, $P < 5 \times 10^{-4}$; c, $P < 5 \times 10^{-5}$; d, $P < 5 \times 10^{-6}$; and e, $P < 5 \times 10^{-9}$ (compared with control).

Previous reports of mouse cloning have often used embryonic stem (ES) cells as nucleus donors (*1*). These cells are nominally attractive subjects for NT studies because they can be genetically manipulated in vitro and are pluripotent. However, offspring derived from the transfer of ES cell nuclei may die perinatally or overgrow (*1*, *7*). By contrast, neonates cloned from somatic cells were indistinguishable from controls. Epigenetic mutations accumulated during culture of ES cells (*8*), not the biological effects inherent to NT, are therefore likely to have been the primary cause of anomalies in ES cell NT clones. In this respect, and because applications such as therapeutic cloning are not envisaged to use ES cells as nucleus donors, ES cells are perhaps a poor model with which to study NT.

References and Notes

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