동물복제 실패원인 규명

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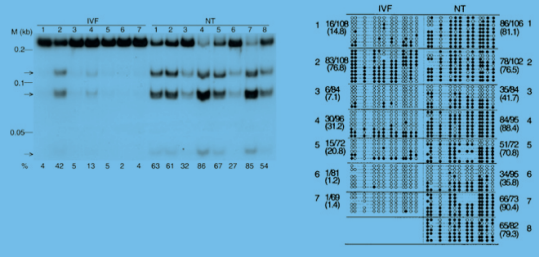
연구개요‥복제 수정란을 자궁에 착상시킬 때 실패율이 95% 이상인데 이러한 동물복제의 실패 원인을 세계 최초로 규명함.

개발내용‥대리모의 자궁에 성공적으로 착상된 복제 배아 가운데, 출산 뒤까지 정상적으로 자라는 경우는 매우 일부분이고, 나머지는 유산, 기형, 급사증후군, 거대체중증후군 등으로 정상 출산이 불가능함.

‥동물복제 실패의 주요 원인은 복제 수정란의 게놈(유전체) 중 특정 염기서열에 '메틸기'(methylation)라는 화학적인 꼬리표(tag)가 복제과정 중에도 그대로 남아 있기 때문으로 나타났음. 포유동물은 세포가 분화할 때, 게놈의 특정 염기서열에 '메틸기'라는 화학적인 꼬리표가 달라붙음. 하지만 정상적인 초기배 발생을 거치는 동안 수정란에서는 이러한 꼬리표가 없어지는데, 이를 탈메틸화(demethylation)라고 함. 그런데 복제 수정란의 경우에는, 무슨 이유에서인지 이러한 탈메틸화가 일어나지 않고, 메틸기가 그대로 남아 있음.



개별 복제수정란의 DNA 메틸화 분석 사진

활용사례 / 효과‥네이쳐 제네틱스(Nature genetics)지에 논문이 발표됨.

‥300회 이상(2010.1.10. 현재) 인용되어 연구의 임팩트가 적지 않음을 증명하였으며 세계적으로 후성유전적 재편의 중요성에 대해서 인식 시킴으로서 동분야의 연구를 진흥에 기여함.

letter

Aberrant methylation of donor genome in cloned bovine embryos

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Despite recent successes in cloning various animal species, the use of somatic cells as the source of donor nuclei has raised many practically relevant questions such as increased abortion rates, high birth weight and perinatal death[1–3]. These anomalies may be caused by incomplete epigenetic reprogramming of donor DNA. Genome-wide demethylation occurs during early development, 'erasing' gamete-specific methylation patterns inherited from the parents[4–8]. This process may be a prerequisite for the formation of pluripotent stem cells that are important for the later development[9]. Here, we provide evidence that cloned bovine embryos may have impaired epigenetic reprogramming capabilities. We found highly aberrant methylation patterns in various genomic regions of cloned embryos. Cloned blastocysts closely resembled donor cells in their overall genomic methylation status, which was very different from that of normal blastocysts produced ***in vitro*** or ***in vivo***. We found demethylation of the ***Bov-B*** long interspersed nuclear element sequence in normal embryos, but not in cloned embryos, in which the donor-type methylation was simply maintained during preimplantation development. There were also significant variations in the degree of methylation among individual cloned blastocysts. Our findings indicate that the developmental anomalies of cloned embryos could be due to incomplete epigenetic reprogramming of donor genomic DNA.

To monitor the epigenetic reprogramming process that acts on a differentiated genome transplanted to enucleated oocyte, we analyzed genomic methylation patterns. We first characterized two kinds of donor fetal fibroblasts maintained in two different growth

We analyzed genomic DNA of in vitro-fertilized (IVF) or cloned (nuclear transfer; NT) embryos for methylation status of the same region. We found a large difference in methylation status using AciI restriction analysis (Fig. 2a); in contrast to the satellite sequence of IVF blastocysts, which was considerably undermethylated (9%), that of NT was heavily methylated (65%), like that of donor cells (72%), indicating abnormal methylation of satellite sequences in NT embryos. Both parthenogenetic (3%) and in vivo-derived blastocysts (5%) also had hypomethylated satellite sequences, like the IVF blastocysts. To rule out the possibility that the satellite region had already undergone a demethylation process before being remethylated at the blastocyst stage, we also examined earlier-stage NT embryos (Fig. 2b,c). The satellite sequences were heavily methylated in NT-derived morulae (63%) and four- to eight-cell embryos (42%). Thus, the methylation status of satellite sequences does not change during cleavage of clones from the methylation level of the donor cells. On the contrary, hypomethylation was noticeable in IVF- (10%) or in vivo-derived morulae (6%), and in four- to eight-cell embryos (14%). The satellite region was moderately methylated in metaphase II-stage oocytes (35%) and considerably undermethylated in sperm (3%), as in mouse sperm[5]. The same DNA region of fertilized one-cell eggs showed 12% methylation, a value that is not very different from the composite methylation value (19%) of the two parental genomes. Because of its initial hypomethylation status, the satellite sequence did not show a demethylation process during cleavage of IVF embryos.