Animal Reproduction Science 116 (2009) 213–225

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Hypomethylation trends in the intergenic region of the imprinted *IGF2* and *H19* genes in cloned cattle

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ARTICLE INFO

Article history:
Received 10 December 2008
Received in revised form 27 January 2009
Accepted 3 February 2009
Available online 11 February 2009

Keywords:
Imprinting
Methylation
IGF2
H19
SCNT
Bisulfite sequencing
Cloning
Cattle
Large offspring syndrome

ABSTRACT

Bos taurus is a good model for embryo biotechnologies such as nuclear transfer. However, animals produced from these technologies often suffer from large calf syndrome, suggesting fetal growth dysregulation. The imprinted fetal mitogen *IGF2* is clustered with *H19* and the two genes are co-regulated in humans and mice. Although the allelic expression pattern of *IGF2*/*H19* has been elucidated in agricultural species such as sheep and cattle, the underlying mechanism of their imprinting regulation has not been characterized. Using bisulfite sequencing the methylation status of 44 CpG sites in a CpG rich intergenic region of *IGF2*/*H19* in the liver, brain, lung, kidney and placenta of control calves (produced by conventional breeding). One fragment containing 16 CpG sites was differentially methylated region (DMR), and thus may be important in regulating *IGF2*/*H19* allelic expression.

The DMR in tissues from cloned term calves that either died immediately after birth or were sacrificed due to complications shortly thereafter were examined. There were significant variations in the methylation of this DMR in some of the cloned animals compared to the controls. Most of the observed variations tended toward hypomethylation. The hypomethylation of this DMR in the liver and placenta of clones correlates with the previous observation of abnormal, biallelic expression of the *H19* allele in those clones [Zhang, S., Kubota, C., Yang, L., Zhang, Y., Page, R., O'Neill, M., Yang, X., Tian, X.C., 2004. Genomic imprinting of *H19* in naturally reproduced

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0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2009.02.008

and cloned cattle. Biol. Reprod.] but not with allelic expression of *IGF2* (as determined in this study). These data suggest that this DMR is involved in *H19* allelic expression, but that other mechanisms probably regulate the expression of *IGF2*/*H19*. Contrary to global hypermethylation observed in cloned embryos, putative imprinting control regions can display hypomethylation trends in specific organs of cloned calves.

1. Introduction

Genomic imprinting is an epigenetic phenomenon in placental mammals and certain plants that marks one parental allele of a specific gene for functional non-equivalence. To date over 80 imprinted genes have been identified in mammals (http://www.igc.otago.ac.nz/), many of which are involved in fetal growth regulation. Among these, insulin-like growth factor 2 (*IGF2*) and *H19* belong to a conserved cluster of imprinted genes that are reciprocally imprinted and expressed during embryonic development. The first imprinted gene identified, *IGF2* is a fetal mitogen (DeChiara et al., 1991) and is subjected to complex transcriptional regulation through alternative splicing, polyadenylation, multiple promoter use and genetic imprinting (Soares et al., 1986; Otte et al., 1998). *H19* functions as an untranslated RNA and is one of the most abundantly expressed imprinted genes in embryonic and fetal development, although its function remains unclear (Goshen et al., 1993). In humans and mice, the coordinated regulation of *IGF2*/*H19* transcription is achieved through the use of a common set of tissue specific enhancers located downstream of *H19*. The genomic region between *H19* and *IGF2* contains a differentially methylated region (DMR) and a chromatin insulator, which regulates the binding of the CCCTC-binding factor (CTCF). When the DMR is methylated, which occurs on the paternal allele, CTCF binding is prevented and the insulator is inactivated, thereby repressing transcription of *H19* and promoting activation of the *IGF2* promoter by the enhancers. When the DMR is unmethylated, as is the case with the maternal allele, CTCF binds to the DMR and creates a boundary that prevents the *IGF2* promoters from accessing the enhancers, and *H19* is transcribed (Charalambous et al., 2004). The *IGF2* gene also contains areas of differential methylation, in mice and humans, which interacts with the *H19* DMR to loop the chromatin into active and inactive domains (Murrell et al., 2004). In the proposed model of chromatin looping, the so-called DMR1 (upstream of *IGF2*) on the maternal allele, interacts with the DMR of *H19* to loop *IGF2* into the inactive domain. On the paternal allele the *IGF2* DMR2, located between exons 6 and 7, interacts with the *H19* DMR, looping *IGF2* and *H19* into the active domain, *H19* continues to be silenced by its own methylation.

Although, both *H19* and *IGF2* are imprinted in cattle, as in mice (DeChiara et al., 1991) and humans (Davies, 1994) underlying mechanism(s) of imprinting regulation have not been determined. Cattle produced from somatic cell nuclear transfer (SCNT) often display abnormal imprinting patterns and amounts of *H19* and *IGF2* expression (Yang et al., 2005).

Similar to humans and sheep, several putative CTCF binding sites and several CpG dense regions were found after *in silico* screening of the cattle intergenic region of *IGF2* and *H19*. Because genomic DNA methylation is the most widely studied epigenetic modification of chromatin and the best candidate for genetic imprints (Glenn et al., 1993), methylation patterns of the CTCF binding region in conventionally bred and cloned cattle were compared with known *H19* expression patterns, using the bisulfite genomic sequencing technique. Bisulfite genomic sequencing allows for the analysis of every CpG site of a given fragment of DNA (Clark et al., 1994) and is considered the gold standard for the in depth assessment of methylation patterns.

Large Offspring Syndrome (LOS) and failure to develop to term are observed in many animal species cloned to date, most notably in cloned mice and cattle (Tamashiro et al., 2000). The poor efficiency in producing live and healthy births from SCNT could be due to the differences in epigenetic establishment of imprinted genes between SCNT and conventional reproduction. In somatic cell nuclear transfer, differentiated somatic cells, with pre-existing genetic imprints, are used to generate cloned animals. Contrasting that, gametes participating in conventional reproduction have already under-