行政院國家科學委員會專題研究計畫 成果報告

小鼠 Dlk1-Dio3 區域基因印痕錯亂之病理分析與調控機制之探討

研究成果報告(精簡版)

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小鼠Dlk1-Dio3區域基因印痕錯亂之病理分析與調控機制之探討

Lineage specific regulatory mechanism and consequences of loss of imprinting at the mouse Dlk1-Dio3 imprinted domain

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中文摘要

基因印痕或基因烙印(Genomic imprinting)乃利用上位遺傳(Epigenetic)之調控機制（亦即DNA甲基化與組蛋白之修飾等），造成印痕基因(Imprinted genes)僅由某一親源之染色體表現的自然生理現象。 遂有母源染色體專一表現及父源染色體專一表現之印痕基因之分。基因印痕對幹細胞與再生醫學之重要性，由複製動物最常見之基因印痕失調造成之發育異常，及胚幹細胞基因印痕印記不穩定之現象可見一斑。本文將以小鼠第12對染色體遠端之*Dlk1-Dio3*基因印痕區域為例，探討基因印痕之調控機轉，及基因印痕遭到干擾後所發生之胚胎發育異常的狀況，以及發育中之胚胎與胎盤在基因印痕調控機制之相異之處。瞭解基因印痕以致於整體上位遺傳調控機制，將顯著提升複製經濟動物之效率並提供幹細胞與再生醫學突破性之發展方向。

Abstract

Genomic imprinting is an epigenetic mechanism controlling parental origin-specific gene expression. Its significance in stem cell research and regenerative medicine has been demonstrated in the imprinting-related developmental abnormality in cloned animals, as well as the imprint mark instability in the cultured embryonic stem cells. In this study, we use the 1 Mb model imprinted locus on mouse chromosome 12 to introduce the regulatory mechanism of genomic imprinting as well as the phenotypic consequences of impaired imprinting control. Our data suggests different degree of imprinting control by the imprinting control center, a germ-line derived differentially methylated region, between the embryonic and extraembryonic lineages. Understanding the imprinting control mechanism in developing conceptuses as well as cultured embryonic stem cells will undoubtly facilitate the development of stem cell technology and regenerative medicine.

Background and Aims

Perturbing the parental origin of the distal portion of mouse chromosome 12 causes alterations in the dosage of the imprinted genes and results in embryonic lethality and developmental abnormalities of the embryo and placenta[1,2]. A 1 MB imprinted domain has been identified on distal chromosome 12 that contains three paternally expressed protein-coding genes and a series of maternally expressed non-coding RNAs. An intergenic parental-origin specific differentially methylated region, the IG-DMR, that is unmethylated on the maternally inherited chromosome, is necessary for the repression of the paternally expressed protein coding genes and activation of the maternally expressed non-coding RNAs and its absence causes the 1Mb imprinted domain on the mutant maternal chromosome to behave like the paternally inherited one (**Fig. 1**)[3].

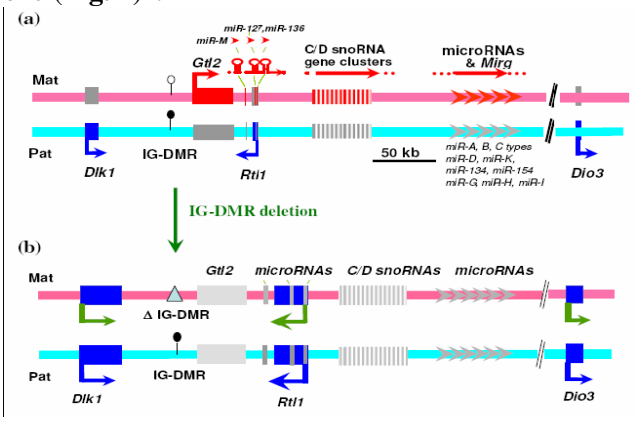

Fig 1. IG-DMR is the imprinting control center for the 1Mb *Dlk1-Gtl2* imprinted locus on mouse chromosome 12[3].

In this study, we (1) characterize the developmental consequences of the epigenotype switch of the *Dlk1-Dio3* domain caused by deletion of the imprinting control region, IG-DMR; (2) study the possible lineage specific imprinting control between the embryos and placentas; and (3) demonstrate the possible *Trans* interaction between the two parental chromosome homologs on *Dlk1-Dio3* domain in homozygous IG-DMR-/- mutants.

Results and Discussions

We compared the phenotypes of the IG-DMR -/+ conceptuses, bearing paternal epigenotype on the 1Mb Dlk1-Dio3 domain, with the phenotypes of paternal uniparental disomy 12 {patDi(12)} conceptues where the entire two chromosome 12s are inherited from the father. The results show that all the embryonic defects described for the uniparental disomy embryos can be attributed to this one cluster of imprinted genes on distal chromosome 12. In contrast, in the placenta, the absence of the IG-DMR has no obvious phenotypic consequence, compared to the placentomegaly and defects in all 3 placental layers identified in patDi(12). The striking discrepancy between the placenta phenotype in PatDi(12) and the IG-DMR-/+ mutant suggests that the IG-DMR may not confer the same epigenetic control over the *Dlk1-Dio3* imprinted domain. This hypothesis has been proven by the incomplete maternal to paternal epigenotype switch in the IG-DMR-/+ placenta. The maternally expressed genes, although somewhat down regulated, are not silenced in placenta as they did in the embryos (**Fig2**).

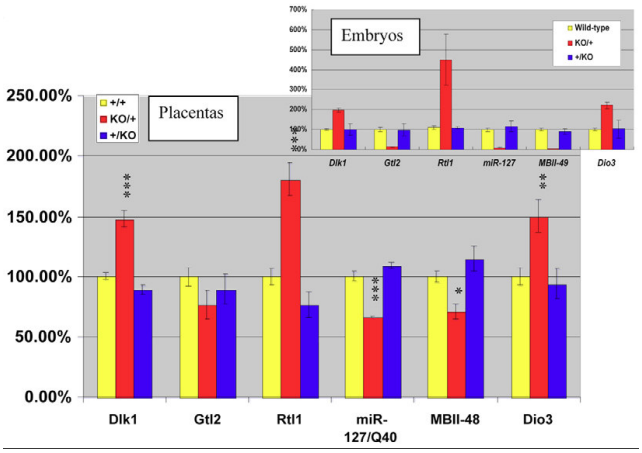

Fig. 2. Expression of imprinted genes in placentas as a consequence of the IG-DMR deletion. For comparison, the inserted panel shows the equivalent expression analysis in E16 embryos.

The different differential DNA methylation pattern between the two embryonic and extraembryonic lineages in the *Dlk1-Dio3* domain (**Fig. 3**) suggest that either there is a different set of imprint marks in the placenta and/or that the placenta possesses a different mechanism of interpreting imprinted marks than in the embryo.

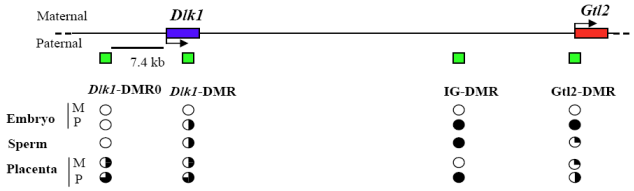

Fig. 3. Summary of the methylation status of the Dlk1-Gtl2 domain in embryos, sperms and placentas. White and black circles represent unmethylated and fully methylated regions, respectively. One, two and three quarters-filled circles represent alleles methylated by approximately 25%, 50% and 75%, respectively. M, maternal allele; P, paternal allele.

Although the paternal transmission of the IG-DMR deletion (IG-DMR+/-) did not cause significant phenotype or defective imprinted gene expression, the homozygous IG-DMR-/- mutants have better viability and occasionally survive till adulthood, suggesting possible *trans*-interactions between the mutant maternal and paternal chromosomal homologs. The incomplete silencing of maternally expressed non-coding RNAs is likely the consequence of this proposed *trans*-activity (**Fig.4**).

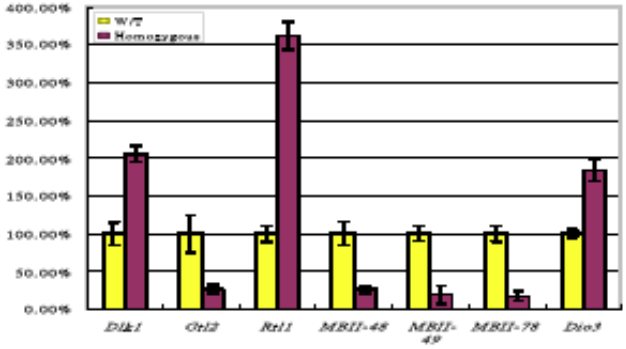

Fig 4. The expression levels of the imprinted genes in homozygous IG-DMR deleted embryos at E16 compared to the wild-type littermates (normalized as 100%).

Conclusion

The implications of lineage-specific and *trans*-allele imprinting control mechanism derived from this study explains some developmental defects in cloned animals and can provide new direction for increasing animal cloning efficiency.

References

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Lin, S.P, *et al* (2007). TSSRC conference paper

出席國際學術會議心得報告

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計畫名稱	小鼠 Dlk1-Dio3 區域基因印痕錯亂之病理分析與調控機制之探討
出國人員姓名 服務機關及職稱	林劭品 國立台灣大學 生物科技研究所 助理教授
會議時間地點	29th Nov-2nd of Dec
會議名稱	■ The 5th Surugadai International Symposium: Genomics and Epigenomics of Human Diseases and Mammalian Development ■ International Genomic Imprinting Workshop 2006 ■ Discussion meeting of Genomic imprinting studies of the Dlk1-Dio3 locus
發表論文題目	Phenotypic consequences of Imprinting control element deletion from the Dlk1-Gtl2 locus.

一、參加會議經過

It was indeed a very intensive 3 meetings combined trip. Through the 7am-11pm daily discussion, I managed to talk to most of the speakers in order to keep abreast of all the new developments in the field of genomic imprinting and the epigenome in diseases and development. In addition, the ongoing study of the current NSC project was presented in the workshop and attracted lots of enquiries. We have also put up an effective discussion meeting concerning this collaborative project of imprinting study on Dlk1-Dio3 locus among my lab, Dr. Ferguson-Smith's lab in UK and Dr. Jerome Cavaille's lab in France. We have exchanged all the updated data and carefully planned the future experiments. Moreover, we also set up the framework for a manuscript from this project, which was later accepted in *Development* journal.

二、與會心得

It was really rewarding to learn that 6 of the presentations from this Imprinting Workshop were directly related to our research work imprinting control and phenotypic consequences of Dlk1-Dio3 imprinting disorder. Three talks were actually extended from my PhD work; including using the imprinting center knock out mice I generated, to produce bi-maternal live offspring (which was later published in Nature Biotechnology. In addition, my collaborative work with Dr. Deborah Bourc'his was also presented. My name was therefore mentioned a lot. Dr. Bourc'his and I have also set up some ground work as how to publish the "DNA methyltransferase 3-like deficient imprint-free embryo work". We have also set up more related collaborative research projects. In general, this has been an extremely fruitful trip concerning upgrading the epigenetic teaching and research work that I am conducting in Taiwan.