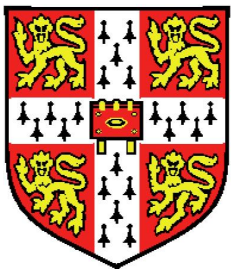
Evolutionary Landscape of CpG Island Methylation in X Chromosome Inactivation



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Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text.

Mengning Liu

To my dearest mum, for giving me unconditional love and the curiosity genes...

Abstract

Dosage compensation in mammals depends upon X Chromosome Inactivation (XCI), the transcriptional silencing of one X in female cells. In human and mouse, CpG island methylation on the inactive X (X_i) has been implicated in the maintenance of XCI and has been used previously to indicate a gene's XCI status. There is evidence that XCI is more complete in mouse than in human, and so I speculated that CpG island methylation on the X_i might also be more extensive in the mouse. In marsupials, by contrast, the small amount of available evidence points to absence of methylation on the X_i. I have studied the methylation of CpG islands on the X chromosomes of human, mouse and opossum in order to provide further evidence for these suggested species differences.

Ninety-one human genes, including several thought to escape from XCI, were assayed together with the mouse orthologues for 52 of these genes. Female and male genomic DNA was digested with *Msp*I or its methylation-sensitive isoschizomer *Hpa*II then tested the ability of the DNA to support amplification of PCR products containing multiple *Msp*I/*Hpa*II sites. Only the three known mouse escapees tested (*Eif2s3*, *Ddx3x*, *Utx*) were hypomethylated in the female, compared to 13 genes in human. This is consistent with the suggestion that gene

silencing is more effective in the mouse. Furthermore, the assay indicated that partial methylation is common among the human genes but rare in the mouse. Bisulphite sequencing of CpG islands from eight human-mouse orthologous pairs has confirmed this difference.

The restriction-PCR assay was then applied to 37 X-linked genes in opossum and CpG island hypermethylation was found to be rare on the female X chromosomes. However, for at least six genes, there was a greater level of methylation in the female sample, which was subsequently confirmed by bisulphite sequencing.

Results from this study support the view that CpG island methylation as a maintenance mechanism for XCI is common in the eutherian mammals. The lower level of methylation in human than mouse is consistent with the suggestion that escape from XCI is associated with a failure to maintain the inactive state. If this is correct, a high level of escape from XCI in marsupials might be anticipated. This study has also provided the first evidence of a possible role of CGI methylation in marsupial XCI.

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