



Epigenetics: Genomic Imprinting and Cancer

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Changes in gene expression that cannot be explained by changes in DNA sequence are known as epigenetic changes. Genomic imprinting is an epigenetic phenomenon whereby some of our genes are expressed from one parental allele with the reciprocal silencing of the other allele. Because imprinted genes are present as both an active and an inactive gene sequence in the same nuclear environment they are an excellent model system to study epigenetics.

Imprinted genes as heritable epigenetic models for cancer predisposition.

Aberrant imprinting of the Insulin-like Growth Factor 2 (*IGF2*) gene is part of the aetiology of the congenital growth and cancer predisposing disease Beckwith Wiedemann Syndrome (BWS, OMIM#130650), as well as various pediatric (Wilms tumour, rhabdomyosarcoma, hepatoblastoma) and adult cancers (Cooper et al., *Eur. J. Hum. Genet.* 2005; 13:1025-23; Murrell, *ScientificWorldJournal* 2006; 6:1888). *IGF2* is normally expressed exclusively from the paternal allele and is over-expressed in many cancers, presumably due to loss of imprinting which is accompanied by DNA methylation changes at the locus. Up to 10% of normal individuals have constitutive loss of *IGF2* imprinting and it has been proposed that these individuals may be predisposed to developing colorectal cancer (Cui et al., *Science* 2003; 299:1753).

We have initially focused on the following specific questions:

- (1) Are the epimutations that lead to loss of imprinting in congenital disease similar to those found in cancer?
- (2) Are the epimutations found in cancer constitutionally

present prior to cancer or are they acquired after cancer?

Children with Beckwith Wiedemann Syndrome are predisposed to Wilms tumours specifically when they have methylation differences within the *H19* gene (Cooper et al., *Eur. J. Hum. Genet.* 2005; 13:1025-23). To answer the first question we examined constitutive and tumour specific methylation patterns of the *IGF2* and *H19* genes in syndromic Wilms tumour patients (i.e. with congenital loss of imprinting such as Beckwith Wiedemann Syndrome) and non-syndromic Wilms tumour patients. Our results show that Wilms Tumour patients with loss of *IGF2* imprinting have similar epigenetic mutations regardless whether they have a congenital imprinting defect or not. Interestingly, Beckwith Wiedemann patients had distinctly different epigenetic mutations associated with loss of *IGF2* imprinting in the tumours and the germline (Murrell et al., *PLoS ONE* 2008; 3:e1849). Thus epimutations in congenital disease and cancer are not identical.

To answer the second question we retrospectively measured the prevalence of *IGF2* hypomethylation in a nested sample set from the EPIC (Easton et al., *Am. J. Hum. Genet.* 1993; 52:678) cohort of peripheral blood DNA samples. Our cohort contained 800 DNA samples that had been collected from individuals 2–4 years prior to diagnoses of colorectal or breast cancer plus 800 age matched controls. Hypomethylation of *IGF2* has been reported to be associated with LOI in colorectal cancer (Cui et al., *Cancer Res.* 2002; 62:6442) and if constitutive hypomethylation of *IGF2* is a predisposition to cancer, then we would expect to find a high prevalence of *IGF2* hypomethylation in either or both of the cancer populations.

Our results indicated that somatic loss of *IGF2* methylation increased with age, but did not predispose to either colorectal or breast cancer. The occurrence of *IGF2* hypomethylation in tumours from colorectal cancer was 80% and actually higher than the prevalence of biallelic *IGF2* expression. These results suggest that *IGF2* hypomethylation

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precedes loss of imprinting in colorectal cancer and is possibly a diagnostic biomarker of cancer rather than a surrogate marker for loss of imprinting (Ito et al., *Hum. Mol. Genet.* 2008; 17:2633). Epimutations in cancer are therefore likely to be specific to the cancer and not constitutive.

The molecular mechanisms that lead to epigenetic changes in gene expression during cancer.

Aberrant methylation of imprinted genes in cancer occurs through impairment of the maintenance mechanisms rather than germ line establishment. Differentially methylated regions (DMRs) of imprinted genes are resistant to genome wide reprogramming during early development, but are easily erased during germ cell development.

Our next two questions are:

- (1) What are the specific features of different types of DMRs that determine susceptibility to demethylation in one context and hypermethylation in another?
- (2) What are the mechanisms whereby maintenance of imprinting breaks down in somatic cells?

Cancer cells have genome wide hypomethylation and CpG island specific hypermethylation, but the mechanisms whereby these changes occur is not known. We have developed a pyrosequencing assay for up to eight CpGs in 80 DMRs associated with 40 imprinted genes. These DMRs have been well characterised in terms of histone modifications and DNA methylation and the effects that these epigenetic modifications have on the expression of the associated imprinted genes. The DMRs also represent a variety of regulatory elements such as promoters, enhancers, insulators, repeat-associated CpG-rich elements and CpG islands. In most cases it is also known whether the DMRs are established in the maternal/paternal germline or acquired somatically and whether they contain binding motives for CTCF or various transcription factors. We have begun to identify specific imprinted DMR profiles for prostate cancer, breast cancer and colorectal cancer cell lines and are gathering data regarding the sensitivity of various DMRs to methylation changes.

Higher order chromatin looping structure may be important in maintaining genomic imprinting. In mice different chromatin loops are formed on the maternal and paternal alleles at the *IGF2-H19* locus. These loops place the *IGF2* gene in different chromatin domains, such that on the paternal allele the *IGF2* gene is in an active domain while on the maternal allele the gene is moved into a silent domain (Murrell et al., *Nat. Genet.* 2004; 36:889). The zinc finger protein CTCF mediates the regulation of *IGF2* imprinting (reviewed in: Lewis and Murrell, *Curr. Biol.* 2004; 14:R284) and directs the secondary looping structure on the maternal

allele (Kurukuti et al., *PNAS* 2006; 103:10684). CTCF has recently been shown to recruit cohesin to its consensus sites (Parelho et al., *Cell* 2008; 132:422; Rubio et al., *PNAS* 2008; 105:8309; Stedman et al., *EMBO J.* 2008; 27:654; Wendt et al., *Nature* 2008; 451:796). We are addressing whether cohesin also plays a role in maintaining chromatin looping structure of the human *IGF2-H19* locus in normal and cancer cells. Preliminary results indicate that cohesin binding is central to looping on the maternal *IGF2* allele (Figure 1). Depletion of cohesin results in loops being disrupted on the silent maternal allele but not on the active paternal allele. This discovery is a new function for cohesin that is distinct from its role in sister chromatid cohesion. These data provide insight into how loss of imprinting and methylation status at the *IGF2* can become decoupled.

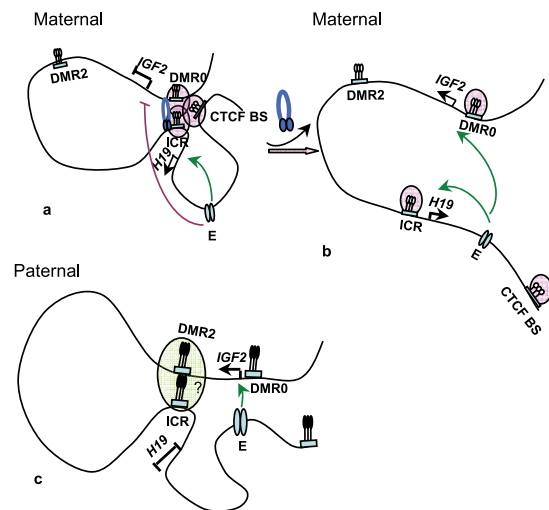


Figure 1. Proposed model for chromatin conformation at *IGF2-H19* locus. (a) Normal state of chromatin conformation at the maternal allele; *IGF2* is enclosed into a Cohesin-CTCF mediated silent loop formed between *DMR0*, *ICR* and an intergenic CTCF binding site (CTCF BS) that prevents the enhancers (E) from accessing *IGF2* promoters. The *H19* promoter can exclusively access the enhancers which enables *H19* expression on the maternal allele. (b) Cohesin depletion destabilises the silent maternal chromatin loop allowing the enhancers to interact with *IGF2* promoters and activate its expression. (c) Normal state of chromatin conformation at the paternal allele. Because of methylation cohesin and CTCF cannot bind, the enhancers interact with *IGF2* promoters and drive transcription. Methylation mediated interaction between a second DMR (*DMR2*) in the *IGF2* gene and *ICR* is proposed to facilitate enhancer-promoter interaction. White lollipop – unmethylated CpGs; black lollipop – methylated CpGs; pink circle – CTCF; blue ring – cohesin; light green circle – putative complex mediating *ICR-DMR2* interaction.

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