Igf2 imprinting in development and disease

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ABSTRACT Igf2 is one of the first imprinted genes discovered and occupies a centre stage in the study of imprinting. This is because it has dramatic effects on the control of fetal growth, it is involved in growth disorders and in cancer, it interacts with products of other imprinted genes, and its imprinting status is under complex regulation in a cluster of tightly linked imprinted genes. Here we review briefly the key features of Igf2 imprinting in normal development and in disease, and hope to show what a fascinating subject of study this gene and its biology provides.

KEY WORDS: imprinting, DNA methylation, fetal growth, mouse development, genetic disease

Role of Igf2 in placenta and fetus

The mouse (and other mammalian) Igf2 gene encodes a single polypeptide (Fig. 1). This polypeptide is thought to have autocrine (same cell), paracrine (surrounding cells), and endocrine (circulation) actions (Efstratiadis, 1998; see below). The IGF-II ligand signals through the IGF1 receptor and the Insulin receptor, and is also bound by the IGF2 receptor (which does not transduce any signal). The biological action of IGF-II is further modulated by IGF binding proteins in serum and tissues. Various effects of IGF-II in vitro on cell proliferation and apoptosis are documented, however, its precise roles in growth in vivo are not fully established (Efstratiadis, 1998). A recent analysis suggests effects on both dry weight and wet weight, but less on DNA content, suggesting roles in cell and tissue reorganisation as well as cell proliferation (Gardner et al., 1999).

Igf2 transcripts are primarily produced in mesodermal, endodermal and extraembryonic tissues, and different enhancers are largely responsible for this tissue specificity (see below). The three promoters P1–P3 are transcribed in all these tissues, whereas promoter PO is active specifically in the placenta (Moore et al., 1997). Although there is some potential for translational differences between these transcripts, they are all thought to be translated (Nielsen et al., 1999). While Igf2 begins to be transcribed shortly after implantation first in extraembryonic tissues, and then throughout mesodermal and endodermal tissues in postimplantation embryos (Lee et al., 1990), the action of gene expression on growth is only seen from E13 onward (Efstratiadis, 1998). Since this is also the case for some of the other components of the IGF/INS pathway (IGF-1, IGF1R), it is likely that critical components of the signalling pathways are only assembled after this point. Both under and overexpression of Igf2 has dramatic and apparently dose dependent effects. Fetuses (and their placentae) completely lacking IGF-II are 40% growth retarded at birth (60% of normal size) and are apparently normally proportioned dwarfs (De Chiara et al., 1990). Fetuses lacking Igf2 expression in endoderm (liver, gut, etc.) through knockout of the endoderm specific enhancers located downstream of H19 (Fig. 3) are 80% of normal size at birth and are also apparently normally proportioned, which may attest to the importance of endocrine IGF-II action (Leighton et al., 1995). The endoderm enhancer KO mice also show that lowered Igf2 expression limited to the fetus (with normal levels in the placenta) has an effect independent of the placenta (Leighton et al., 1995). Overexpression of Igf2 can increase size at birth up to 160% (Sun et al., 1997), and size at E17 up to 200% (Eggenschwiler et al., 1997), in a manner dependent on IGF-II dosage. High levels of overexpression also lead to various malformations and intrauterine death (Eggenschwiler et al., 1997; Sun et al., 1997; see below). Individual organs can be enlarged in proportion to their Igf2 levels, suggesting autocrine or paracrine control (Sun et al., 1997). The issue of paracrine or endocrine control is not fully resolved, but new genetic models including the endoderm enhancer KO and a mutant that we are studying in collaboration with B. Cattanach (Harwell) which lacks Igf2 expression in mesoderm (K. Davies et al., unpublished), may allow further clarification.

While IGF-II action can affect the growth of the fetus (through signalling via IGF1R and INSR), it has a particularly unique and important action in the placenta. Thus, while Igf1 and Igf1r KO mice show no effect on normal size placenta, all manipula-
The importance of placental IGF-II production is confirmed in another system, in which chimeric conceptuses were constructed that lacked IGF-II in trophoblast and hence labyrinthine and spongiotrophoblast of the placenta, but the fetus was normal genotype (Gardner et al., 1999). The effects on fetal and placental weights on E16.5 were identical to the ones recorded in the placental specific KO (M. Constancia et al., unpublished; Gardner et al., 1999).

The observation that genes for fetal growth factors which are particularly important for placental growth and development are imprinted (Igf2, Igf2r), whereas those whose roles are limited to the fetus are not (Igf1, Igf1r, Insr), raises the question of whether the primary selection for imprinting comes from action of growth factors in the placenta with a direct effect on nutrient transfer from mother to offspring. It also raises the possibility that there might be differences in mechanistic aspects of imprinting in placenta and fetus.

IGF-II interacts with the products of other imprinted genes (Igf2r, H19, probably Grb10, Miyoshi et al., 1998) and it will be interesting to see whether other imprinted growth related genes also interact with the IGF pathway, in particular perhaps in the placenta.

**Mechanisms of Igf2 imprinting**

The *Igf2* gene is part of a cluster of imprinted genes on distal chromosome 7 in the mouse, with a homologous cluster on the syntenic region on chromosome 11p15.5 in the human (Paulsen et al., 1998; Fig. 2). The *lgf2* gene is paternally expressed (De Chiara et al., 1991). A number of sequences that are important for *Igf2* imprinting and expression have now been defined. *Igf2* imprinting is under regional control particularly influenced by the neighbouring maternally expressed *H19* gene (Leighton et al., 1995b; Ainscough et al., 1997; Brannan and Bartolomei, 1999). The influence of the *H19* control sequences apparently also extends to *Ins2*, but probably not further to other genes in the cluster (Caspy et al., 1998). Whether other genes or control sequences in the cluster have an influence on *Igf2* or *H19* imprinting or expression is currently uncertain.

Two sequences have been identified so far with clearly defined roles in vivo in imprinting and expression of *Igf2/H19*. The first is a set of enhancers called the endoderm enhancers which are located 3' of the *H19* gene (Fig. 3). Knockout of these enhancers abolishes expression of *H19* (on the maternal chromosome) in mainly endodermal tissues such as liver and gut, and of *Igf2* (on the paternal chromosome) in the same tissues (Leighton et al., 1995a). This establishes that these enhancers are shared by both genes. So, how is the action of the enhancers limited to either gene on different parental chromosomes? Upstream of *H19* is located a differentially methylated region (DMR) with paternal methylation and maternal undermethylation (the paternal methylation and maternal undermethylation (the paternal methy-
alation also extends into the H19 promoter and gene body and is thought to silence the paternal gene copy). When this region is deleted, H19 is no longer methylated on the paternal chromosome, and is now expressed. Similarly, the maternal copy of Igf2 is expressed from a chromosome with the deletion (Thorvaldsen et al., 1998). The currently favoured interpretation is that this DMR represents some sort of chromatin insulator or boundary element which when methylated allows access of the Igf2 promoters to the H19 enhancer (Thorvaldsen et al., 1998; Webber et al., 1998). In its unmethylated state, the element represents a closed boundary which prevents activation of the Igf2 promoters by their enhancers. Interestingly, a specialised non-histone type chromatin structure has recently been shown to exist on the maternal (unmethylated) DMR region (Hark and Tilghman, 1998; Szabo et al., 1998; Khosla et al., 1999). The DMR consists of various elements, including a direct repeat structure and a potential silencer (Lyko et al., 1997), whose detailed functions in vivo are unclear at present. The knockout of the DMR also results in a reduced level of Igf2 expression on a chromosome that also expresses H19 (and vice versa) suggesting that the promoters of both genes also compete for the shared enhancers (Thorvaldsen et al., 1998). As mentioned earlier, enhancers for lineages other than endoderm have so far not been found, but we are studying a radiation induced mutant in which Igf2 expression is abolished in mesodermal tissues (K. Davies et al., unpublished).

How the Igf2 promoters access the distant enhancers (or are prevented from doing so presumably by an unmethylated DMR) is unclear. It is remarkable that strong DNase I hypersensitive sites are present in Igf2 promoters and upstream on both parental chromosomes (Sasaki et al., 1992; Feil et al., 1995). The Igf2 gene itself also has three DMRs (Fig. 3) but whether these have functions independent of H19 and its DMR/enhancers or in conjunction with these is currently not known (Sasaki et al., 1992; Feil et al., 1994; Moore et al., 1997). Knockouts in DMR1 and DMR2 have been prepared, however, and are under study, so that some of these answers should soon emerge.

The H19 DMR seems to carry a ‘germline methylation imprint’ in the sense that the sperm copy is methylated, the oocyte copy is not, and these methylation patterns are inherited through all stages of development (Olek and Walter, 1997; Tremblay et al., 1997), except in germ cells where they are switched as appropriate. DMR1 in Igf2 (which was the first DMR to be discovered, Sasaki et al., 1992) is apparently not differentially methylated in germ cells but becomes so soon after fertilisation (Shemer et al., 1996). DMR2 is differentially methylated in germ cells, and loses this methylation in the early preimplantation embryo, which then becomes re-established later on (Oswald et al., unpublished). Thus, whether these sequences carry germline imprints is not totally clear, however, it should be borne in mind that there could be epigenetic memory systems other than methylation that interact with the methylation system (Pickard et al., unpublished). The methylation patterns at Igf2 DMRs 0–2 are not independent of the H19 DMR/gene, but whether this is because of altered expression of Igf2 as a consequence of deletion of the H19 DMR/gene, or whether there are elements that are responsible for regional establishment and spreading of the ‘epigenotype’ is currently not clear (Forné et al., 1997; Moore et al., 1997).

Although other genes in the cluster also have DMRs, and the cluster as a whole displays asynchronous DNA replication during the cell cycle as a global feature (Kitsberg et al., 1993), other regional or local control elements have so far not been defined (but see below for some hints). In any event, the H19–Igf2 system of genes is an excellent model system for the study of germline imprints and regional control of imprinting. The mechanistic details of this system are under intense scrutiny and discussion (Constancia et al., 1998; Reik and Walter, 1998; Surani 1998; Tilghman 1999; Brannan and Bartolomei, 1999), but presentation of these details goes beyond the scope of the current review.

**Altered IGF2 expression in disease**

There are now a number of disease situations in which levels of IGF2 expression are altered (Ward 1997; Morison and Reeve, 1998; Feinberg 1999). IGF2 expression is often increased and this
Loss of imprinting of *Igf2* has been described as an isolated somatic event, largely associated with various pediatric and adult cancers, or an embryonic or germline event associated with the overgrowth and cancer syndrome Beckwith-Wiedemann syndrome (see below). Many cancers (but with notable exceptions) show LOI of *IGF2* (Feinberg, 1999). Such LOI could either be causally involved in tumour initiation or growth, or be an epi-phenomenon because of altered maintenance of DNA methylation and potentially imprinting in tumours. To our knowledge, a large scale analysis of the stability or otherwise of imprints in tumours has not yet been carried out. However, there is evidence that overexpression of *IGF2* can indeed lead to tumour initiation or progression (Christofori et al., 1994; Bates et al., 1995).

In terms of mechanisms, the best studied system is Wilms’ tumour, a childhood tumour of the kidney. A large proportion of tumours with *IGF2* LOI also have maternal methylation of the *H19* gene (Moulton et al., 1994; Steenman et al., 1994). This is consistent with the model described above in which methylation of the *H19* DMR would allow access of *IGF2* promoters to the shared enhancers. This hypermethylation of *H19* can sometimes be observed in adjacent healthy tissues, suggesting that it is an early, or even predisposing event, in tumourigenesis (Moulton et al., 1994; Okamotomo et al., 1997). However, no mutational mechanisms leading to this epigenetic switch have been identified, and to our knowledge, this type of event has not been observed in rare familial cases of the disease. Hence, it is conceivable that this epigenetic switch could be the result of ‘epimutation’. Although this could arise randomly, a proposal has been made for a mechanism by ‘methylation transfer’ from the methylated paternal to the unmethylated maternal *H19* DMR (Bestor and Tycko, 1996). Confirmation of this mechanism awaits analysis in a genetic model.

Germline or early embryonic alterations in *IGF2* imprinting could lead to LOI in many cells of the developing organism (Dean et al., 1998). This seems to be the case in the fetal overgrowth syndrome, Beckwith-Wiedemann syndrome, in which the majority of patients show apparent LOI at least in fibroblasts cultured from their skin (Weksberg et al., 1993; Reik et al., 1995; Reik and Maher, 1997). In mouse models, the majority of the symptoms of the disease can indeed be attributed to overexpression of *Igf2* (Sun et al., 1997; Eggerschwiler et al., 1997). The mechanisms by which LOI arises, and the molecular pathogenesis in BWS patients in which there is no LOI of *IGF2*, are apparently complex but all molecular abnormalities detected so far are linked to 11p15.5 (Fig. 4; Reik and Maher, 1997). The majority of BWS patients are sporadic, and familial cases are rare. A number of sporadic cases show maternal methylation of *H19*, with associated LOI of *IGF2* (Fig. 4; Reik et al., 1995). Surprisingly, other patients with LOI of *IGF2* show normal imprinting of *H19* (Joyce et al., 1997). A minority of BWS patients have maternally inherited translocations in 11p15.5, with several breakpoints in KvLQT1 (Lee et al., 1997a), or further centromeric outside of the present clusters (Mannens et al., 1996). In two families in which this could be analysed, translocation was associated with LOI of *IGF2* (Brown et al., 1996; Smaililich et al., 1999), but the mechanism of this is unknown. In another series of sporadic patients, LOI of the KvLQT1 antisense gene occurred at a high frequency (Fig. 4; Lee et al., 1999; Smaililich et al., 1999) which in some cases was associated with LOI of *IGF2* but in others not. Hence, currently it
is unclear whether mutational (translocation) or likely epigenetic (LOI of antisense gene) events in the KvLQT1 region interfere with IG2F imprinting/translation. If they do not, this would reinforce the notion of a bipartite structure of the cluster, with potentially independent imprinting controls (Caspary et al., 1998). However, strikingly, it would also lend support to the previous suggestion that alterations in linked imprinted genes in the cluster could result in similar phenotypes, perhaps because these genes interact in common physiological pathways (Reik and Maher, 1997). Indeed, the only point mutations discovered so far in BWS patients have been found in the cell cycle inhibitor p57Kip2/CDKN1c (Fig. 4; Hatada et al., 1996; Lee et al., 1997b; O’Keefe et al., 1997; Lam et al., 1999), and these do not seem to be associated with LOI of IG2F. These mutations are more frequent in familial cases than in sporadic ones (Lam et al., 1999), whereas the epigenetic alterations in H19 or KvLQT1 antisense are exclusive to sporadic patients. The suspicion is therefore that the majority of BWS patients have epimutations—in either H19 or KvLQT1 antisense—rather than genetic mutations.

Conclusions

The study of imprinting and phenotypic effects of the IG2F gene, its imprinted neighbours in the cluster, and its interacting components encoded elsewhere in the genome, will continue to be a fascinating subject of imprinting research. Because growth and cell proliferation are such central themes in imprinting, progress in understanding the regulation of IG2F will continue to lead to key insights into the biology and pathology of growth and cell proliferation.

Acknowledgements

Work in the authors’ laboratory is supported by BBSRC, MRC, CRC, MAFF, HFSP and DFG.

References


