Genomic imprinting and cancer

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Abstract

Genomic imprinting is the phenomenon by which individual alleles of certain genes are expressed differentially according to their parent of origin. The alleles appear to be differentially marked during gametogenesis or during the early part of development. This mark is heritable but reversible from generation to generation, implying a stable epigenetic modification. Approximately 25 imprinted genes have been identified to date, and dysregulation of a number of these has been implicated in tumour development. The normal physiological role of many imprinted genes is in the control of cell proliferation and fetal growth, indicating potential mechanisms of action in tumour formation. Both dominant and recessive modes of action have been postulated for the role of imprinted genes in neoplasia, as a result of effective gene dosage alterations by epigenetic modification of the normal pattern of allele specific transcription. The aim of this review is to assess the importance of imprinted genes in generating tumours and to discuss the implications for novel mechanisms of transforming mutation.

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The appearance of partial penetrance in pedigrees of children subject to developmental tumour predisposition in Beckwith-Weidemann syndrome[1] and to hereditary glomus tumours[2] in similar pedigrees had long been puzzling, as penetrance appeared to be most complete for transmission through one of the two parental sexes. However, such a pattern of inheritance can be reconciled with a model in which the familial mutation is present in an imprinted gene, and penetrance is only seen when a recessive lesion is inherited from the parent whose allele would normally be expressed, rendering the individual effectively nullizygous for a tumour suppressor locus. Alternatively, the observed pattern might be explained by a dominant tumour growth enhancing factor, whose normally suppressed allele is activated by mutation.

The development of hereditary glomus tumours or paragangliomas only occurs when the mutant locus is inherited paternally.[3] The disease maps to two loci on chromosome 11, 11q13.1 and 11q22.3–11q23.3, both of which appear to be imprinted, although the genes have yet to be identified.[2 4 5]

The first concrete evidence that imprinted genes might be involved directly in tumorigenesis came from the observation of an absolute bias (95–100%) in the loss of 11p15 maternal alleles, as indicated by loss of heterozygosity (LOH) in Wilms' tumours and embryonal rhabdomyosarcoma.[6 7] Selective maternal allele loss has now been documented in other childhood tumours—adrenocortical carcinoma and hepatoblastoma—as well as in adult cancers of the breast, bladder, ovary, kidney, and testis (table 1). Consistent LOH for a particular chromosome or locus is a strong indication that

Table 1 Alterations in imprinted genes in various human cancers

Cancer	*Pref. maternal 11p15 LOH*	*IGF2 LOI*	*IGF2 mRNA increase*	*H19 LOI*	*H19 mRNA decrease*	*H19 mRNA increase*	*$p57^{KIP2}$ LOI*	*$p57^{KIP2}$ mRNA decrease*	*6q LOH*	*IGF2R mutations*	*References*
Wilms' tumour	+	+	+	+	+		+	+	ND	ND	7–15
Adrenocortical carcinoma	+	+	+	ND	+		ND	+	ND	ND	16–19
Rhabdomyosarcoma	+	+	+	−	+		ND	ND	ND	ND	6, 18, 20–23
Hepatoblastoma	+	+	ND	−	+		ND	ND	ND	ND	24–26
Hepatocellular carcinoma	+	+	ND	+		+	ND	ND	+	+	18, 27–31
Breast cancer	+	+	+	ND	ND	ND	ND	ND	+	+	18, 32–36
Lung cancer	+	+	+	+		+	ND	ND	+	ND	18, 33, 37–41
Colorectal cancer	ND	+	+	−	ND	ND	ND	ND	ND	+	42, 43
Leiomyosarcoma	ND	+	ND	−	ND	ND	ND	ND	ND	ND	44
Testicular germ cell cancer	+	+	ND	+	ND	ND	ND	ND	ND	ND	18, 45, 46
Renal cell carcinoma	ND	+	+	−	ND	ND	ND	ND	+	ND	47, 48
Endometrial cancer	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	42
Kidney clear cell sarcoma	ND	+	+	ND	ND	ND	ND	ND	ND	ND	49
Choriocarcinoma	ND	+	+	+		+	ND	+	ND	ND	50–53
Cervical carcinoma	ND	+	ND	+	ND		ND	ND	+	ND	54, 55
Oesophageal cancer	ND	+	+	+		+	ND	ND	ND	ND	56, 57
Bladder cancer	+	ND	ND	ND		+	ND	ND	ND	ND	18, 58
Acute myeloid leukemia	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	18, 59
Gastric cancer	ND	+	+	−	−		ND	ND	+	ND	42, 60–62
Glioma	ND	+	ND	−	ND	ND	ND	ND	+	ND	63, 64
Ovarian carcinoma	ND	−	+	ND	ND	ND	ND	ND	+	ND	48, 65, 66
Neuroblastoma	ND	−	ND	−	ND	ND	ND	ND	ND	ND	67

The categories used are: pref. maternal 11p15 LOH, preferential maternal 11p15 loss of heterozygosity; IGF2 LOI, IGF2 loss of imprinting; IGF2 mRNA increase; H19 LOI, H19 loss of imprinting; H19 mRNA increase or decrease; $p57^{KIP2}$ LOI, $p57^{KIP2}$ loss of imprinting; $p57^{KIP2}$ mRNA decrease; 6q LOH, loss of heterozygosity on chromosome 6q; IGF2R mutations, mutations detected in the IGF2R gene. Classifications are based on more than one positive report in each category and the primary publications are referenced.

there might be a tumour suppressor gene located within the smallest common deficiency. Tumour suppressors are usually inactivated sequentially on both alleles according to Knudson's "two hit" hypothesis.[68] In the case of maternally expressed tumour suppressor genes, the "first hit" is the imprinting or inactivation of the paternal allele and the preferential maternal LOH observed is equivalent to the "second hit". Thus, the effective constitutive hemizygosity, resulting from epigenetic silencing of one allele, is the normal state, rather than the result of a predisposing genetic mutation, as originally envisaged by Knudsen and Strong.

In the case of 11p15.5, the parental bias of allele loss in tumours suggests either the loss of a maternally active tumour suppressor gene, or the acquisition of a paternally expressed growth promoting factor through duplication of the expressed copy. In support of the former hypothesis, three groups have transferred subchromosomal fragments from this region into rhabdomyosarcoma and Wilms' tumour cell lines and assayed the potential of these fragments to suppress in vivo tumorigenicity in nude mice.[69–71] These combined studies demonstrate the presence of a potential tumour suppressor gene(s) within a 500 kilobase (kb) region of 11p15.5. Alternatively, or additionally, it is possible that preferential loss of maternal alleles is driven by the selective advantage of acquiring a double dose of a paternally active factor by gene duplication, which promotes tumour cell growth. Although the identity of the putative tumour suppressor at 11p15.5 is as yet unknown, a strong candidate for a tumour growth promoting factor is insulin-like growth factor II (IGF-II); the gene encoding this factor maps to 11p15.5 and lies within a cluster of imprinted genes.

Growth promoters at 11p15

The role of the IGF system in tumour growth has been reviewed recently.[72] The first observation of raised IGF-II concentrations in a neoplastic situation was made in a series of developmental tumours, where expression of the IGF2 gene was greatly enhanced (up to 50 fold) with respect to the age matched normal tissue, and comparable to that seen in earlier phases of development.[8] The observation that a fetal growth related pattern of growth factor expression is not extinguished during tissue differentiation suggests that this is an example of "oncofetal" gene expression. More recently, the observation of enhanced IGF2 expression in a wide variety of adult tumours suggests that there might be a clear selective advantage in high levels of expression of this gene. Interestingly, concentrations of IGF-I are raised much more rarely, although the type 1 receptor, through which both factors act, is often present at increased concentrations in tumours.[73] However, the latter is not imprinted in humans or mice.[74] Genetic ablation of the type 1 receptor has been shown to abrogate the effects of a variety of transforming agents, such as simian virus 40 (SV40) T antigen[75] and other oncogenes, suggesting that, although the IGFs do not act as classic transforming growth factors, their action might be essential to allow the full transforming potential of other oncogenes. Accumulating evidence suggests a role for IGF-II in cell survival and suppression of apoptosis, a mechanism of action supported by experiments in vivo.[76] Activation of IGF2 expression seems to be an early event in some systems, and has been shown to contribute towards tumour growth by reducing apoptosis during the preneoplastic phase of pancreatic β cell transformation in a transgenic model.[77] Similarly, a high degree of expression has been shown in nephrogenic rests and in preneoplastic smooth muscle lesions, indicating that the time window during which IGF2 expression becomes raised might differ between tumour types.[9 44]

IGF2 is an imprinted gene, as first demonstrated in the mouse following gene knockout,[78] and then in the human by direct analysis.[79 80] It is expressed almost completely from the paternal allele (> 95%) during development and, therefore, breakdown in the normal suppression of the maternal allele by loss of imprinting (LOI), or duplication of the active paternal allele, are likely candidates for the mechanism increasing IGF2 expression; both effectively doubling the available transcriptional template. The first tumour in which this was observed was Wilms' tumour, where LOI for IGF2 and/or the adjacent gene H19 was reported in the majority of cases, most of which were early onset, implying that LOI of IGF2 was an early event in Wilms' tumorigenesis.[10 11 81] Significantly, in the tumour predisposing Beckwith-Wiedemann syndrome, IGF2 imprinting is constitutively relaxed, indicating that enhanced transcription might in itself predispose towards tumour growth, without being sufficient to initiate tumours alone,[82 83] consistent with a scenario in which IGF2 expression acts to affect cell survival and, consequently, predisposes to the accumulation of further transforming mutations.

However, it is becoming clear that there is a discrepancy between LOI and an overall rise in IGF2 mRNA.[84 85] For example, LOI of IGF2 is seen in Ewing's sarcoma but is not always associated with increased expression of IGF2 mRNA.[86] Increased expression of IGF2 in Wilms' tumours and some hepatoblastomas is not always dependent on loss of genomic imprinting.[67 85 87] It is also clear that LOI is not a prerequisite, even within one tumour category.

In addition, there are quantitative considerations to take account of because the general increase in IGF2 mRNA concentrations associated with LOI are usually greater than the expected 100% for a doubling of available template—for example, in leiomyosarcoma.[88] This suggests either that additional non-imprinting dependent mechanisms are operating, or that in some way interallelic processes become active, and that these allow transsensing of active transcription and upregulate the process in both copies.

Although IGF2 is an excellent candidate for the key imprinted growth stimulatory gene in

this part of the genome, loss of imprinting at IGF2 is by no means a prerequisite for tumour formation. IGF2 lies within a cluster of genes that are regulated coordinately by imprinting, including H19 and $p57^{KIP2}$ (CDKN1C). Therefore, it could be that the tumour suppressor and/or promoter activity suggested by genetic studies is the result of mutations in other closely linked genes, which are themselves additional candidates for contribution towards the neoplastic state.

Tumour suppressors at 11p15

H19

H19 maps to within 200 kb of IGF2, yet it is imprinted in the opposite direction, being expressed exclusively from the maternal allele.[89] Alterations in H19 imprinting status and expression have been reported in a number of cancers (table 1) and it was initially proposed to be a tumour suppressor gene, based on two types of observations. Primarily, expression of H19 was found to be reduced, or even extinguished, in a proportion of Wilms' tumours and other tumours.[12 13] Secondarily, forced expression experiments initially supported a direct tumour suppressive effect of H19.[90] The mechanism by which H19 might act as a tumour suppressor gene is not clear because the gene encodes an untranslated RNA of unknown function.[91] Transfection of an H19 expression construct into the malignant G401 and rhabdomyosarcoma tumour cell lines generated altered cellular morphology, retarded growth, and reduced tumorigenicity[91]; however, discrepant results have been reported by other workers.[71]

The reciprocal pattern of imprinting to that of IGF2, suggested to be caused by enhancer competition between promoters of the two genes,[92] frequently breaks down when IGF2 LOI occurs, both in neoplastic and non-neoplastic situations, indicating that H19 extinction is, as discussed above for IGF2 overexpression, not in itself necessary for the development of cancer. In support of this, Reid *et al.* found that H19 was expressed in several non-tumorigenic cell lines, but also in significant amounts in tumorigenic cell lines.[71] Moreover, a discordantly high level of expression of H19 has also been reported in a number of human cancers including lung, oesophageal, and bladder carcinoma (see table 1 for full list). Recent data linking tumour progression with H19 expression confirms the suggestion that H19 LOI is either an epiphenomenon associated with locus specific or more global changes in imprinting control, or an example of oncofetal gene expression.[27] In either case, a direct, causal link with tumour formation now looks unlikely.

$p57^{KIP2}$

$p57^{KIP2}$ is a member of the p21 family of cyclin dependent kinase inhibitors (CDKIs), which inhibit cell cycle progression by binding to the cyclin dependent kinases (CDKs) responsible for the G1/S transition.[93 94] However, $p57^{KIP2}$ is the only member of the CDKI families (which also include INK proteins) that is known to be imprinted, being expressed predominantly from the maternal allele.[95 96] Because human $p57^{KIP2}$ maps to 11p15.5 and is maternally expressed, it is possible that it might be a tumour suppressor, with preferential maternal LOH resulting in a loss of $p57^{KIP2}$ expression.

It is intuitively easy to envisage how the loss of $p57^{KIP2}$ activity, a negative regulator of the cell cycle, could be responsible for the increased proliferative advantage of tumours in which it is inactivated. In addition, a recent study indicated that $p57^{KIP2}$ interacts with proliferating cell nuclear antigen (PCNA) a cofactor for the δ subunit of DNA polymerase, suggesting that this might be another pathway (in addition to CDK inhibition) that prevents cell proliferation.[97]

A reduction in $p57^{KIP2}$ expression has been noted in some Wilms' tumours[14 15] but, as yet, its expression and imprinting status have not been examined in a wide range of neoplasias. In two recent studies, the paternal allele of $p57^{KIP2}$ was found to be expressed in five of five[98] and 10% of Wilms' tumours examined.[99] Paradoxically, this was associated with an overall decrease in mRNA concentrations in all tumours in one study,[99] suggesting that if $p57^{KIP2}$ does act as a tumour suppressor, the mechanism of transcriptional downregulation is dominant over the expected effects of loss of imprinting.

$P57^{KIP2}$ mutations have not been found in any of the tumours analysed to date (which have included soft tissue sarcomas, Wilms' tumours, liver, and lung cancers)[37 99–101] and in only ~ 10% of patients with Beckwith-Weidemann syndrome (these mutations were present constitutively and were not associated with tumour development).[102–104] In addition, $p57^{KIP2}$ expression was found in tumorigenic clones of the G401 cell line (as mentioned above for H19), indicating that at least in this assay it is unlikely to be responsible for suppression of tumorigenicity.[105] Thus, it is still unclear whether $p57^{KIP2}$ is the elusive 11p15.5 tumour suppressor gene.

Recently identified candidate tumour suppressor genes

Subchromosomal fragment complementation assays have allowed the mapping of the gene(s) responsible for tumour suppressor function telomeric to D11S601,[71] partially overlapping with a 3 Mb region responsible for retardation of tumour growth.[70] The region of overlap (~ 500 kb) has been shown recently to contain a number of novel candidate tumour suppressor genes. At least two of these are known to be imprinted (IPL/TSSC3 and BWR1A), whereas the other genes have not yet been characterised fully (TSSC1, TSSC2 and BWR1B).[106–108] IPL/TSSC3 is homologous to the gene encoding the TDAG51 protein, which is involved in Fas mediated apoptosis, and BWR1A shows strong homology to the genes encoding the tetracycline resistance efflux proteins.

BWR1A was screened for mutations in various tumour cell lines (breast and lung carcinomas, rhabdomyosarcomas, Wilms', and rhabdoid tumours) and in patients with

Beckwith-Weidemann syndrome.[108] Two mutations were detected in two patients (a 111 bp insertion in a breast cancer and a point mutation in a rhabdomyosarcoma). Because the mutation frequency was quite low, it is possible that BWR1A might be silenced by epigenetic inactivation, as is seen with H19, and possibly $p57^{KIP2}$. Indeed, this might also be the case for the new 11p15.5 genes, such as GOK, the loss of expression of which has been implicated recently in rhabdomyosarcoma.[109] The mechanism by which it is repressed was suggested to be epigenetic because genetic alterations were not detected.

It is possible that these genes are regulated coordinately by an "imprinting centre", as has been postulated for the Prader-Willi and Angelman syndromes.[110] Mutations that disrupt the imprinting centre could affect downstream genes resulting in loss of imprinting/loss of expression in the various 11p15.5 genes, as is seen in a large number of human cancers (table 1). It will be interesting to see if mutations (genetic or epigenetic) are found in the other candidate genes, particularly TSSC3, given the possibility that it might function in apoptosis.

Other tumour suppressors

IGF2R AND WT-1

The insulin-like growth factor type 2 receptor gene (IGF2R) is imprinted in the mouse, and is also expressed exclusively from the maternal allele.[111] However, in humans it is polymorphically imprinted, and most of the population express IGF2R from both parental alleles.[112] The chromosomal region to which IGF2R maps (6q26–27) frequently shows LOH in certain tumours (table 1), and it has been shown recently to be mutated in liver, breast, stomach, endometrial, and colorectal cancers.[28 32 42 61] IGF2R is thought to be responsible both for extracellular IGF-II degradation and the trafficking of lysosomal enzymes. Gene ablation in transgenic mice leads to increased circulating concentrations of IGF-II, with concomitant overgrowth during development.[113]

The type 2 receptor is a multifunctional protein, also responsible for the activation of transforming growth factor β (TGFβ), which in its active form can then inhibit growth by binding to TGFβ receptors in some cell types.[114] Thus, loss or mutation of this gene would be predicted to result in an increase in extracellular IGF2 concentrations (stimulating growth) and a concomitant deficiency in activation of latent TGFβ (possibly preventing apoptosis and growth inhibition). Indeed, these predictions have been confirmed recently in gastric and colorectal cancers.[115] In addition, recent evidence that IGF2R is mutated at early stages in liver and breast tumorigenesis supports the prediction that tumours in which IGF2R is mutated have a substantial selective growth advantage.[29]

Similar polymorphic imprinting is seen in the expression of the Wilms' tumour suppressor gene (WT1) located at 11p13.[116] In principle, populations in which WT-1 and IGF2R are imprinted should show an increased tumour incidence, or at least a differential involvement of these genes in the development of neoplasia. In support of this, a recent study reported that the paternal IGF2R allele was silenced in 50% of Wilms' tumours.[117] Although it is difficult to test the consequences for differential tumour susceptibility, responses to environmental factors, such as radiation, might have important consequences for the interpretation of epidemiological data concerning tumour incidence.

Conclusions

It is now becoming increasingly obvious that disruption of imprinting is common in a wide range of human cancers of both embryonal and adult origin. While much of the work to date has involved identification of the imprinted genes involved in cancer, the precise involvement of these genes in the tumorigenesis pathway needs to be elucidated. Importantly, the realisation that some mutations (imprinting centre mutations) might affect the imprinting of a cluster of genes, implies that in some cases concerted action by aberrently expressed growth promoters and suppressors might be important in key rate limiting processes during tumour formation.

Not only might imprinted genes be involved generally in the control of cell proliferation, but they might also constitute members of the same regulatory pathways; consequently, similar pathologies arising from different mutations might simply reflect the epistatic nature of the gene products in the same process. This implies that although we might see very similar phenotypic effects of mutations in this class of gene, the genes actually affected might differ between individual tumours or predisposing syndromes.

The possibility of both epistasis and coordinate regulation greatly complicate the task of interpreting the pathology resulting from dysregulation of imprinted genes, but our knowledge to date concerning the interactions between at least some of these genes suggests that it is precisely these complications that make our understanding of the role of imprinted genes in cancer so important.

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